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ABSTRACTS

BHS Abstracts

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2.1. Coronary blood flow is predominately driven by a backward propagating ‘suction’ wave, which is attenuated in left ventricular hypertrophy

JE Davies¹, ZI Whinnett¹, CH Manisty¹, IS Malik¹, RA Foale¹, K Willson², AD Hughes¹, KH Parker³ and J Mayet¹

¹International Centre for Circulatory Health, St Mary’s Hospital & Imperial College, London, UK; ²Department of Clinical Engineering, Royal Brompton Hospital, London, UK and ³Physiological Flow Laboratory, Imperial College, London, UK

Background: Coronary blood flow peaks in diastole when aortic pressure has fallen. Current models fail to completely explain this phenomenon. We present a new approach – using wave intensity analysis – to explain this phenomenon in normal subjects, and to evaluate the effects of left ventricular hypertrophy (LVH) on the coronary microcirculation.

Methods: Simultaneous pressure and Doppler velocity were measured using intracoronary wires in the left main stem, left anterior descending, and circumflex arteries of 20 subjects (mean age 54 ± 10 years, 13 female) following a normal coronary arteriogram. Coronary wave speed was calculated using a new method we have recently developed and wave intensity analysis then applied.

Results: A consistent pattern of six predominating waves was identified. 94% of wave energy, accelerating coronary blood forward, came from two waves: first a pushing-wave from left ventricular ejection and later a suction-wave from relief of microcirculatory compression. This suction-wave ($18.2 \pm 13.7 \text{ } 10^3 \text{ W m}^{-2} \text{ s}^{-1}$, 30%) was larger than the pushing-wave ($14.3 \pm 17.6 \text{ } 10^3 \text{ W m}^{-2} \text{ s}^{-1}$, 22.3%, $P=0.001$) and was associated with a substantially larger incremental increase in coronary flow velocity (0.51 versus 0.14 m/s, $P<0.001$). In LVH, the suction-wave was decreased (33.1 versus 26.9%, $P=0.01$) and inversely correlated with septal wall thickness ($r=-0.52$, $P<0.02$, Figure 1).

Conclusion: Six waves predominantly drive human coronary blood flow. Coronary flow peaks in diastole because of the dominance of a suction-wave generated by microcirculatory decompression. This is significantly reduced in LVH.

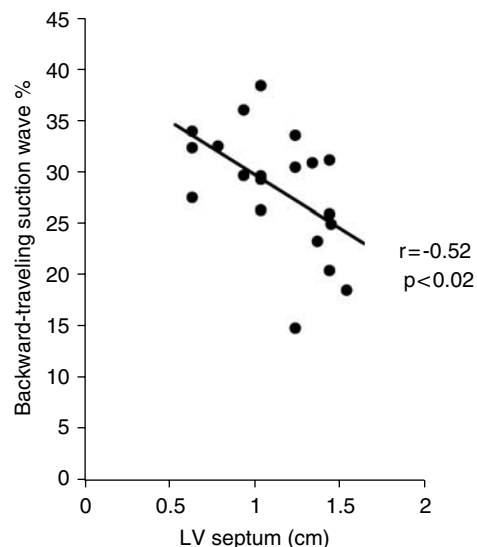


Figure 1 Decrease in backward-travelling suction wave with increasing LVH.

2.2. Ezetimibe and simvastatin both reduce inflammation, disease activity, aortic stiffness and improve endothelial function in rheumatoid arthritis

KM Maki-Petaja¹, AD Booth¹, FC Hall², SML Wallace¹, A Furlong², CM McEniery¹, J Brown³ and IB Wilkinson¹

¹Clinical Pharmacology Unit, University of Cambridge, Cambridge, UK; ²Dept of Clinical Medicine, University of Cambridge, Cambridge, UK and ³Trinity College, University of Cambridge, Cambridge, UK

Background & aims: HMG-CoA reductase inhibitors (statins) have been shown to have anti-inflammatory

and disease modifying properties in patients with rheumatoid arthritis (RA). The aim of this study is to

investigate the effect of simvastatin and ezetimibe on inflammation, disease activity, arterial stiffness and endothelial function in patients with RA and to test our hypothesis that cholesterol lowering per se can improve arterial stiffness and reduce inflammation.

Methods: 20 RA patients received simvastatin 20 mg and ezetimibe 10 mg in a double-blind cross over study. Blood pressure, aortic pulse wave velocity (PWV) and flow mediated dilatation response (FMD) were measured before and after each treatment. Serum inflammatory markers and disease activity were also determined. Data are mean changes \pm s.e.m., and significance was determined using 2-way repeated measures ANOVA.

Results: As expected both ezetimibe and simvastatin significantly reduce total cholesterol (-0.62 ± 0.12

and -1.28 ± 0.11 mmol/l, respectively; $P < 0.0001$). Both drugs significantly reduced CRP (-5.35 ± 2.07 and -5.05 ± 1.41 mg/l; $P = 0.0002$); disease activity (-0.74 ± 0.24 and -0.50 ± 0.18 ; $P < 0.0001$); aortic PWV (-0.69 ± 0.26 and -0.71 ± 0.16 m/s; $P = 0.0012$) and concomitantly, FMD was significantly improved (1.37 ± 0.26 and $2.51 \pm 0.48\%$; $P = 0.0001$). Importantly, only the effect on total cholesterol differed significantly between the drugs ($P < 0.001$).

Conclusions: The present study shows that both ezetimibe and simvastatin reduce inflammatory markers and disease activity to a similar extent in patients with RA. Moreover, aortic PWV was reduced with both drugs and concomitantly, endothelial function was improved. This suggests that cholesterol lowering per se has anti-inflammatory effects and improves vascular function.

2.3. Ambulatory arterial stiffness index (AASI) provides additional prediction of stroke mortality over pulse pressure: the Dublin outcome study

E Dolan¹, E O'Brien¹, L Thijs², Y Li², P McCormack¹, J Staessen² and A Stanton¹

¹ADAPT Centre, Beaumont Hospital, and Molecular and Cellular Therapeutics, Royal College of Surgeons in Ireland, Dublin, Ireland and ²Study Coordinating Centre, Laboratory of Hypertension, Hypertension and Cardiovascular Rehabilitation Unit, Department of Molecular and Cardiovascular Research, University of Leuven, Leuven, Belgium

Background: We hypothesize that the dynamic relationship between systolic blood pressure (SBP) and diastolic blood pressure (DBP) over twenty-four hours, provides a measure of arterial stiffness. The objective of this study was to determine the additional predictive value of the ambulatory arterial stiffness index (AASI), over and above 24-h mean

database of normotensive subjects. After a median follow-up of 5.3 years there were 151 stroke deaths. Twenty-six of these deaths were among 2727 patients with normal daytime ambulatory pressure ($< 135/85$ mm Hg). A Cox proportional-hazard model was created using SAS software.

Results:

		Unadjusted HR	Adjusted HR	Fully adjusted HR*
All patients (151 deaths)	AASI (1 SD)	1.71 (1.47–1.97)	1.23 (1.04–1.45)	1.21 (1.01–1.45)
	Dichotomized AASI (0, 1)	3.06 (2.01–4.64)	2.49 (1.64–3.80)	2.42 (1.58–3.72)
Normotensive (26 deaths)	AASI (1 SD)	2.46 (1.75–3.45)	1.87 (1.24–2.82)	1.81 (1.18–2.78)
	Dichotomized AASI (0, 1)	7.14 (3.17–16.0)	5.99 (2.61–13.7)	5.60 (2.41–13.0)

arterial pressure (MAP) and pulse pressure (PP) for stroke mortality.

Methods: At baseline, whilst not on antihypertensive medication, 11,291 patients (5326 male, mean age 54.6 years) underwent ambulatory BP monitoring. Using all blood pressure readings from each individual we plotted DBP against SBP, and calculated the regression slope. AASI was defined as one minus this regression slope. We also dichotomized AASI using the upper boundary of the 95th prediction interval in relation to age using an international

Hazard ratios (HR) were adjusted for sex, age, MAP, body mass index, smoking, diabetes mellitus, and a history of cardiovascular disease. *Fully adjusted HRs were additionally adjusted for PP.

Conclusions: This novel dynamic index of arterial stiffness, which can be readily determined from ambulatory blood pressure recordings, is strongly and independently associated with stroke risk, particularly amongst normotensive subjects.

2.4. Optimising the analysis of stroke prevention trials (OAST-P): assessment using ordered rather than dichotomous outcomes?

CM Geeganage, PMW Bath, LJ Gray, T Collier and S Pocock

The University of Nottingham, Nottingham, UK

Background: Vascular prevention trials mostly count dichotomous events, e.g. stroke/no stroke. Ordered categorical outcomes based on stroke outcome (e.g. fatal/non-fatal/no stroke) could be more powerful statistically.

Methods: Published summary data from positive or negative (not neutral) antithrombotic, lipid or blood pressure lowering, or carotid endarterectomy vascular prevention trials were identified. The efficiency of nominal and ordinal analyses were compared using Friedman 2 way ANOVA with multiple comparison procedures.

Results: Many trials did not publish sufficient event data to create ordered stroke outcomes. Data from 37 trials (153,346 patients) were analysed for stroke as fatal/non-fatal/no stroke. The analyses differed ($P < 0.0001$) and were ranked (most efficient first); bootstrap of mean, Mann Whitney *U*-test, robust rank test, ordinal regression, Cochran-Armitage

(Chi-square for ordered data, 2×3), *t*-test, Chi-square 2×2 on stroke/no stroke, Chi-square 2×3 (non-ordered data), Chi-square 2×2 on death/alive, median test. When comparing the tests, bootstrap and MWU were more efficient than the other tests. Similar findings were obtained for analyses of myocardial infarction (fatal/non-fatal/no event), and combined vascular events. 4 level (stroke: fatal/severe non-fatal/mild/no event) and 5 level (stroke: fatal/severe non-fatal/mild/TIA/no event) analyses appear to be even more efficient.

Conclusions: Vascular prevention trials should use 3 or higher level ordered outcomes in preference to dichotomous events since these give additional information on treatment effects by severity of stroke, and will permit more efficient ordinal analyses thereby potentially resulting in smaller trials and the calculation of smaller numbers-needed-to-treat.

2.5. Could differences in wave reflection explain the results of the ASCOT trial?

CH Manisty, JE Davies, ZI Whinnett, DP Francis, A Zambanini, S Curtis, SMcG Thom, A Hughes and J Mayet

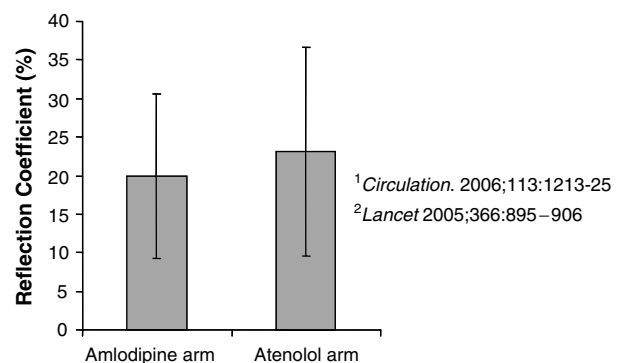
International Centre for Circulatory Health, St Mary's Hospital & Imperial College, London, UK

Background: Treatment differences in central blood pressure were recently demonstrated in the CAFÉ trial¹, and may provide an explanation for the benefits unrelated to brachial blood pressure seen in the ASCOT trial². Changes in wave reflection contribute to the pathophysiology of hypertension, and different pharmacological agents are likely to have differing effects on it.

Wave intensity analysis enables us to discriminate and measure reflection waves directly. Using this tool, we are therefore able to assess whether differences in wave reflection between the treatment arms could explain the differences in central pressures seen in the CAFÉ study, and hence the endpoints of ASCOT.

Method and Results: We measured wave reflection using non-invasive wave intensity analysis in the carotid artery, of 255 subjects (mean age 64 ± 7.7 years) in the Hypertension Associated Cardiovascular Disease (HACVD) substudy of the ASCOT trial.

The magnitude of wave reflection was significantly lower in the amlodipine-perindopril arm ($n = 120$) than the atenolol-bendroflumethiazide arm ($n = 135$) – reflection coefficient 20 versus 23% ($P = 0.02$) – (Figure 1).



There was no difference in the timing of wave reflection between subjects in the two treatment groups ($P = 0.86$), when corrected for the length of systole.

Conclusions: Wave reflection is reduced to a greater extent in subjects in the amlodipine-perindopril arm than the atenolol-bendroflumethiazide arm. The greater wave reflection in the atenolol-bendroflumethiazide group may explain not only the higher central blood pressures seen in the CAFÉ trial, but also the significantly higher incidence of endpoints in this limb of the ASCOT trial.

2.6. Influence of breastfeeding duration, maternal lipidaemia, and blood pressure on aortic pulse wave velocity in young children: The Manchester children's cardiovascular health study

N Bansal¹, I Gemmell¹, P McElduff², V Charlton-Menys¹, A Vyas¹, PN Durrington¹, PE Clayton¹ and JK Cruickshank¹

¹University of Manchester, Manchester, UK and ²Hunter New England Population Health, Newcastle, Australia

Introduction: Maternal hypercholesterolaemia during pregnancy has been linked with increased aortic pathology in childhood, while breastfeeding is associated with lower blood pressure (BP) in adult life. Their effects on vascular function in young children, particularly on arterial stiffness are unknown.

Aim: We tested the hypothesis that breastfeeding duration and higher maternal and child serum LDL-C and triglycerides would be associated with increased arterial stiffness, as measured by aortic pulse wave velocity (aPWV), in preschool children.

Methods: 57 children, whose mothers were studied at 28 weeks gestation, were followed from birth to 2–4 years of age. Measures of BP, aPWV, anthropometry (height, weight, skinfold thickness), and serum lipids (TC, LDL-C, HDL-C and triglycerides) were taken. A maternal questionnaire, completed at the visit, provided information on the incidence and duration of breastfeeding. APWV was measured

using two continuous wave doppler ultrasound probes insonating the aortic arch and bifurcation.

Results: In a univariate longitudinal model analysis, breastfeeding duration was positively associated with aPWV ($\beta = 0.01$, $P = 0.05$); however, maternal and child lipids, BP, and pulse pressure were not significantly associated with aPWV. After adjustment for pulse pressure, age, gender, ethnicity, weight, length, and total skinfolds, duration of breastfeeding was positively associated with aPWV ($\beta = 0.02$, 95% CI 0.008 to 0.03, $P = 0.001$).

Conclusions: Our data suggest that increased duration of breastfeeding is positively associated with aPWV, and thus increased aortic stiffness in early childhood. This is consistent with previous findings where increased duration of breastfeeding has been associated with decreased brachial artery distensibility in adulthood. Maternal lipids and child BP do not appear to be associated with arterial stiffness at this early age.

3.1. Regional and cellular localisation of the epithelial sodium channel subunits in human adrenal, and evidence for a role in regulating aldosterone response to sodium ion fluctuation

J Wang, MJ Brown, RE Kuc and R Foo

University of Cambridge, Cambridge, UK

The epithelial sodium channel (ENaC) is an important regulator of sodium reabsorption and blood pressure. We have previously demonstrated the presence of ENaC mRNA and protein in human adrenal zona glomerulosa (Wang *et al.* BHS Abstract 5.6, 2005). Functional studies with the H295R human adrenocortical cell line revealed an increase in aldosterone secretion in response to the ENaC inhibitor, amiloride. To elucidate further the potential role of adrenal ENaC, we have investigated localisation of its three subunits both among zones of normal human adrenal, and within individual adrenal cells. We also conducted a further experiment in H295R cells to determine whether aldosterone secretion would be increased by the amiloride analogue, EIPA. This selectively inhibits the sodium/hydrogen antiporter (NHE), hitherto considered the channel responsible for adrenal sodium transport. Immunohistochemistry was performed on three adrenals (normal and tumour) removed from patients with Conn's adenomas, using commercial rabbit or goat primary antisera to each of the ENaC

subunits, and peroxidase labelled secondary antisera. The same primary but immunofluorescent secondary antisera were used for confocal imaging of the ENaC subunits in both H295R and cells cultured from fresh human adrenals. All three ENaC subunits were found in zona glomerulosa of human

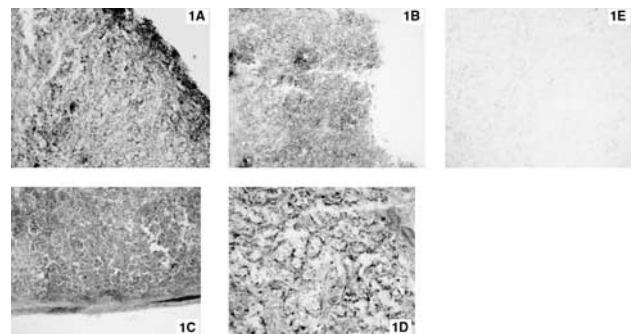


Figure 1A, 1B, 1C=ENaC α , β , γ , respectively, in human adrenal; 1D=ENaC α in kidney cortex; 1E=no primary antibody (adrenal).

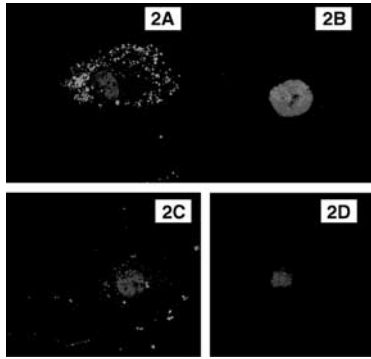


Figure 2 2A, 2B, 2C=EnaC α , β , γ , respectively, in single adrenal cells, double-stained with DAPI (nuclear stain); 2D=DAPI alone.

adrenals (Figure 1) and Conn's tumours, but immunocytochemistry showed distinct cellular localisation, with ENaC α found predominantly within the cytoplasm, ENaC β within the nucleus and ENaC γ around the nuclear envelope (Figure 2). Some migration of channels towards cell the membrane was stimulated by incubation for 24 h with aldosterone. The pharmacological experiment

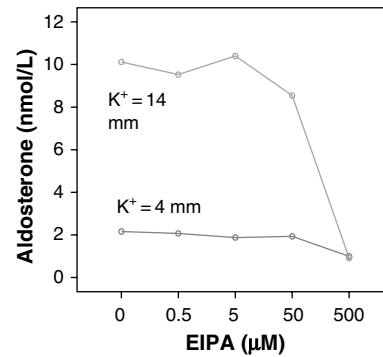


Figure 3

(Figure 3) showed that EIPA, unlike amiloride, does not stimulate aldosterone secretion; at high concentrations, both drugs inhibit secretion completely. These results confirm a potential role for adrenal ENaC as the mediator of aldosterone response to changes in plasma Na^+ , with the intriguing possibility that Na^+ sensing occurs in the nucleus.

3.2. Human p22phox polymorphism (-675A/T) is associated with systolic blood pressure and carotid intima-media thickness

MU Moreno Zulategui¹, G San Jose¹, A Fortuno¹, O Beloqui², J Diez³ and G Zalba¹

¹Division of Cardiovascular Sciences, Centre for Applied Medical Research, University of Navarra, Pamplona, Spain; ²Department of Internal Medicine, University Clinic. School of Medicine, University of Navarra, Pamplona, Spain and ³Department of Cardiology and Cardiovascular Surgery, University Clinic, School of Medicine, University of Navarra, Pamplona, Spain

Oxidative stress is highly relevant in cardiovascular pathophysiology. The NADPH oxidases are the main sources of superoxide anion in phagocytic and vascular cells. The NADPH oxidase p22phox subunit and its genetic variants are implicated in cardiovascular disease.

Our previous results identified the p22phox -675A/T polymorphism, which associated with essential hypertension. Now we corroborated this association in 680 subjects. Genotypes were determined by RFLP and allelic discrimination. The TT genotype prevalence was higher in hypertensives (Normotensives, $n=258$, TT:83.7%, AT:15.9%, AA:0.4%; Hypertensives, $n=422$, TT:89.8%, AT:10.2%, AA:0%; $P<0.05$). We further validated this association in an independent population (153 normotensives and 199 never treated hypertensives). Interestingly, in this population TT hypertensives displayed higher systolic blood pressure levels (TT:152 \pm 1 mm Hg, TA/AA:141 \pm 3 mm Hg; $P<0.05$).

To assess the functionality of the -675A/T polymorphism we measured phorbol-stimulated NADPH oxidase activity, by chemiluminescence with lucigenin 5 μM , in phagocytes from 100 subjects. Results showed that subjects carrying the -675-TT genotype exhibited greater ($P<0.05$) NADPH oxidase activity than -675-AT and AA subjects. Besides, ultrasound-determined carotid intima media thickness, surrogate marker of atherosclerosis, was greater ($P<0.05$) in TT subjects compared with -675-AT and AA individuals. Finally, *in vitro* reporter gene studies demonstrated a functional role of this polymorphism in human p22phox promoter activity.

In conclusion, the human p22phox -675A/T polymorphism is a genetic marker in essential hypertension; it may help discriminate subjects that are under greater oxidative stress and atherosclerotic risk. These results support the relevance of the human p22phox gene and the role of the NADPH oxidase system in cardiovascular disease.

3.3. Novel potent vasoconstrictor action of kisspeptins in human coronary artery and umbilical vein

EJ Mead, RE Kuc, JJ Maguire and AP Davenport

University of Cambridge, Cambridge, UK

The orphan G-protein coupled receptor KISS1 (GPR54) has been paired with products of the KiSS-1 metastasis suppressor gene, kisspeptin (KP)-54, KP-14, KP-13 and KP-10. KPs have been identified as inhibitors of cancer metastasis and as having a role in placentation, processes requiring angiogenesis. Our aim was to determine the role of KPs in human vasculature.

RT-PCR showed remarkably discrete localisation of KISS1 to smooth muscle of developmentally related human tissues umbilical vein (UV), aorta and coronary artery (CA), the latter of which are intriguingly prone to atherosclerotic plaque formation. Fluorescence dual labelling immunocytochemistry additionally detected co-localisation of KISS1 and KPs to atherosclerotic plaques of CA and to vascular endothelial cells. Reversible, saturable, specific and high affinity binding of our novel ligand [¹²⁵I]KP-13 was detected in SM of human aorta (K_D 0.2 ± 0.03 nM, B_{max} 7.65 ± 0.95 fmol/mg protein). *In vitro* studies on isolated rings of human CA ($n=3$) and UV ($n=3$) identified a previously undescribed potent vasoconstrictor action of KP-10, KP-13 and KP-54 in these tissues (Table 1).

We have discovered, for the first time, that KP are potent vasoconstrictors of human UV and CA, with the response in CA being more potent than that of Angiotensin II. Furthermore we have detected specific localisation of KISS1 in vessels prone to atherosclerotic plaque formation. This discovery suggests a previously undescribed role for KPs in cardiovascular disease.

Table 1 Endothelium independent vasoconstriction of human coronary artery and umbilical vein

Vessel	Peptide	pD_2	E_{max}	n
Coronary Artery	KP-10	7.89 ± 0.24	33.74 ± 17.04	3
	KP-13	8.66 ± 0.88	35.05 ± 7.94	3
	KP-54	8.86 ± 1.11	25.69 ± 5.52	4
Umbilical Vein	KP-10	8.42 ± 0.24	24.98 ± 4.15	8
	KP-13	8.43 ± 0.88	28.40 ± 8.62	3
	KP-54	8.93 ± 0.44	35.71 ± 5.77	8

Data are expressed as mean \pm s.e.m. n : number of patients in which responses were recorded.

3.4. Do pharmacological interventions reduce oxidative stress in a model of genetic hypertension?

HHC Koh-Tan, D Graham, CA Hamilton, MW McBride and AF Dominiczak

University of Glasgow, Glasgow, UK

Objectives: Two pharmacological intervention studies were performed to dissect the causal role of oxidative stress in the pathogenesis of hypertension, focusing on two major sites of action – kidney and vessels.

Methods: Reversal study: 16-week-old male SHRSP were treated orally with olmesartan (20 mg/kg/day), hydralazine plus hydrochlorothiazide (H+H; 16 mg/kg/day) or vehicle for 4 weeks. Prevention study: 8-week-old male SHRSP were treated orally with olmesartan, H+H or vehicle for 8 weeks. WKY were treated with vehicle only ($n=6-9$ /group). Blood pressure (BP) was measured weekly by tail-cuff plethysmography. Superoxide production was measured by lucigenin chemiluminescence. Vascular and renal gene expression was measured by quantitative RT-PCR.

Results: Drug treatments in both studies reduced BP in the SHRSP to WKY levels. Superoxide production in renal cortex (nmoles/min/ μ g protein) was

reduced by olmesartan treatment in both reversal (Control: 2.64 ± 0.60 ; Olmesartan: 1.62 ± 0.51 , $P=0.002$) and prevention (Control: 1.25 ± 0.04 ; Olmesartan: 0.90 ± 0.13 , $P=0.016$) studies. Significantly lower *Gstm1* expression (fold-change) in SHRSP kidney (1.00 ± 0.11) compared to WKY (6.67 ± 0.42 ; $P=0.008$) was not improved by either drug treatment. Vascular expression of p22phox (fold-change) was elevated in SHRSP (Reversal: 1.00 ± 0.24 ; Prevention: 1.00 ± 0.14) compared to WKY (Reversal: 0.47 ± 0.09 ; Prevention: 0.72 ± 0.07 , $P=0.056$) and was reduced by olmesartan treatment in both reversal (0.76 ± 0.10) and prevention (0.77 ± 0.11) studies.

Conclusion: Angiotensin receptor blocker reduced oxidative stress at least partially via reduction of p22phox expression. The *Gstm* gene family is less consistently influenced by pharmacological interventions due to strain-dependent genetic abnormalities.

3.5. Genetic and transcriptomic analysis of chromosome 1 blood pressure QTL in the spontaneously hypertensive rat

AJ Bingham¹, J Clemitson¹, RJ Dixon¹, S Haines¹, B Patel¹, M Lo², J Sassard², FJ Charchar¹ and NJ Samani¹

¹University of Leicester, Leicester, UK and ²Faculte de Pharmacie, Lyon, France

Introduction and Aims: Chromosome 1 in the rat contains a quantitative trait locus (QTL) with a major effect on blood pressure (BP). We have previously confirmed the effect of this locus on BP by the production of reciprocal congenic strains (WKY.SHR-Sa) and (SHR.WKY-Sa) derived from a cross of the spontaneously hypertensive rat (SHR) with the Wistar-Kyoto rat (WKY). Furthermore, we constructed a congenic substrain (Sisa1) from the SHR.WKY-Sa strain with only a 4.3 Mb introgressed region which also exhibited the BP effect. Our aims were to carry out further genetic dissection of this region and to identify positional candidate genes through transcriptome analysis.

Methods: We fine mapped the QTL region by systemic construction of two mutually exclusive congenic substrains. Genome-wide microarray expression profiling in whole kidney was undertaken to identify differentially expressed genes among the parental SHR, WKY, congenic strains (WKY.SHR-Sa), (SHR.WKY-Sa) and the substrain Sisa1, at both 6 and 24 weeks of age.

Results and Conclusion: We have reduced the congenic segment to 3Mb region by the construction of two strains (Sisa1a/Sisa1b) that break down the Sisa1 region. Only Sisa1a congenic exhibit reduced BP compared to the SHR animals (166.1 ± 2.5 mm Hg, ($n=17$) vs 174.4 ± 1.4 mm Hg ($n=20$), $P<0.001$). We have identified 4 hypertension candidate genes (Spondin 1, Homer-2, Thumpd1 and XP_215009) that mapped within the defined boundaries of the initial BP QTLs on chromosome 1 and that exhibited significant differential expression between the WKY and SHR genotypes, at both 6 and 24 weeks of age. These differentially expressed genes were confirmed by performing quantitative RT-PCR. One of these genes, Spon1 maps within the boundaries of our new minimal congenic region and has not been previously identified as a hypertension candidate gene. The results of this study justify further investigation of these candidate genes and their involvement in BP control in hypertensive models and humans.

3.6. Retinal microvascular responses to hyperoxia/hypoxia and brachial artery flow-mediated dilation are selectively impaired in patients with small vessel and large vessel ischaemic stroke respectively

A Fulton¹, E Dolan², J Moroney¹ and AV Stanton³

¹Department of Neurology, Beaumont Hospital, Dublin, Ireland; ²ADAPT Centre, Beaumont Hospital, Dublin, Ireland and ³Molecular and Cellular Therapeutics, Royal College of Surgeons in Ireland, Dublin, Ireland

Lacunar infarction or small vessel stroke (SVS) and large vessel stroke (LVS) together account for over 80% of all strokes. Endothelial dysfunction is thought to play key roles in the initiation and propagation of macrovascular and microvascular disease. Brachial artery responses to ischaemia, flow mediated vasodilatation (FMD), is a well established measure of large vessel endothelial function. We are currently evaluating retinal microvascular responses to hypoxia and hyperoxia as measures of microvascular endothelial function. The aim of this study was to compare retinal arteriolar reactivity (RAR) and brachial FMD in SVS versus LVS.

One hundred and three patients consecutively hospitalized with SVS or LVS (TOAST criteria) were evaluated. Brachial artery diameter was measured by ultrasound at rest, during reactive hyperaemia, and after sublingual glyceryl trinitrate (GTN). Using

digital photography and operator directed image analyses, average diameter of 7 arterioles, from the superior temporal quadrant of a single eye, was determined at rest, after inhalation of 10% O₂ for 5 min (hypoxia), after inhalation of 100% O₂ (hyperoxia), and after GTN.

Retinal arteriolar dilatation to hypoxia and constriction to hyperoxia were approximately halved in SVS compared to LVS ($P<0.05$). By contrast, brachial FMD was normal in SVS, but was markedly impaired in LVS ($P<0.01$). Amongst all subjects, RAR to hyperoxia and brachial FMD were inversely correlated ($P<0.01$). Brachial artery and retinal arteriolar diameter responses to GTN did not differ with stroke subtype.

Our findings support meaningful pathophysiological differences between SVS and LVS and the validity of pursuing stroke sub-typing.

4.1. Angiotensin II promotes accelerated ageing of human vascular smooth muscle cells via reactive oxygen species generation

KE Herbert, Y Mistry, RA Hastings, AG Stanley, T Poolman and B Williams

University of Leicester, Leicester, UK

Background: Angiotensin II (Ang II) contributes to hypertension, atherosclerosis, vascular remodelling and hypertrophy. Animal models have shown that inhibition of Ang II attenuates ageing effects in cardiovascular tissue by decreasing NAD(P)H oxidase-derived reactive oxygen species (ROS) production. The development of various age-related vascular diseases is characterised by accumulation of senescent vascular cells at sites predisposed to disease. Furthermore, biological ageing, as measured by telomere attrition, is a characteristic of cardiovascular disease. We investigated the hypothesis that Ang II causes senescence of cultured human vascular smooth muscle cells (hVSMCs) via a ROS-initiated signalling mechanism.

Methods: hVSMCs were cultured in the presence of Ang II (10^{-9} to 10^{-6} mol/l) for up to 14 days. DNA damage was assessed by determining DNA single-strand breaks (Comet assay). Lucigenin chemiluminescence was used to detect ROS generation.

Cellular ageing was determined by (i) telomere restriction fragment length using Southern blotting and (ii) cell senescence by senescence-associated β -galactosidase staining.

Results: Ang II induced ROS generation and a rapid increase in DNA strand breaks. DNA strand scission was inhibited by pre-incubation with either the Ang II type 1 receptor (AT1R) antagonist, E3174 or with catalase. 24 h exposure to Ang II caused a dose-dependent increase in cell senescence that was dependent on ROS generation. Ang II exposure also accelerated telomere attrition with a 2.5-fold increase in mean loss of telomere DNA compared to controls.

Conclusion: Ang II-induced ROS generation leads to DNA damage, accelerated hVSMC senescence and telomere attrition. These studies identify a novel mechanism to implicate Ang II in the pathogenesis of human vascular cell ageing and senescence.

4.2. Two WNKs to a better understanding of blood pressure control and electrolyte balance

M O'Reilly, E Marshall, T MacGillvray, M Mittal, W Xue, CJ Kenyon and RW Brown

Centre for Cardiovascular Science, Queen's Medical Research Institute, Edinburgh, Scotland, UK

WNK1 (With-No-K, lysine) and WNK4 are unusual kinases mutated in Gordon syndrome an autosomal dominant, hypertensive, hyperkalaemic disorder; implicating this novel WNK pathway in regulation of blood pressure (BP) and electrolyte balance. Previously we identified full-length (WNK1-L) and short (WNK1-S), kinase-deficient WNK1 isoforms. Importantly WNK1-S is overwhelmingly predominant in kidney. Recent *Xenopus* oocyte studies implicate WNK4 in inhibition of both thiazide-sensitive cotransporter-mediated Na^+ -reabsorption and K^+ -secretion via ROMK, and now suggest WNK4 is inhibited by WNK1-L itself inhibited by WNK1-S.

Here we examine gene expression in this WNK pathway along the nephron in mouse kidney in detail, and how expression changes *in vivo* in response to challenges altering renal electrolyte handling and aldosterone. These studies reveal for

the first time expression of WNK1-S and WNK4 is strongest in distal tubule dropping sharply in collecting duct and with WNK4 also expressed in thick ascending limb (TAL) and the macula densa, extending the spectrum of potential WNK4 targets to TAL transport and NKCC2.

In vivo, this novel WNK pathway responds to chronic changes in dietary electrolytes and aldosterone, with significant upregulation of WNK1-S and WNK4 with high K^+ -intake, and reduction in WNK1-S on chronic lowering of K^+ - or Na^+ -intake and quite different responses in primary and secondary hyperaldosteronism. We reveal a two compartment distal nephron model explaining these findings and Gordons syndrome well with WNK and classic aldosterone pathways responding to drivers from K^+ balance, volume and aldosterone and cross-talk through distal Na^+ delivery regulating electrolyte balance and blood pressure.

4.3. Cardiac growth restriction precedes the onset of cardiac hypertrophy in the hypertrophic heart rat

ER Porrello^{1,2}, CL Curl¹, SB Harrap¹, WG Thomas² and LMD Delbridge¹

¹Department of Physiology, The University of Melbourne. ²Baker Heart Research Institute, Prahran, Melbourne

Cardiac hypertrophy, even in the absence of hypertension, significantly increases cardiovascular risk.¹ The Hypertrophic Heart Rat (HHR) is a normotensive model of cardiac hypertrophy, which displays cardiomyocyte hypertrophy in association with an apparent reduction in myocyte number.² This suggests the possibility of reduced hyperplasia or increased apoptosis during early cardiac development. The aim of this study was to establish a link between neonatal myocardial apoptosis and the developmental onset of cardiac hypertrophy in the HHR. Cardiac ventricles were freshly harvested from HHR and control strain Normal Heart Rats (NHR) at several developmental stages (p2 and 4, 6, 8, 12 wk). HHR cardiac weight indices were considerably smaller than NHR at day 2 (4.33 ± 0.19 vs 5.01 ± 0.08 mg/g), but 'caught-up' to NHR by 4 weeks (5.10 ± 0.15 vs 5.16 ± 0.11 mg/g). By 12 weeks, HHR

hearts were 27% larger than NHR. The incidence of apoptosis was studied by morphological analysis of cultured neonatal cardiomyocytes after 72 h exposure to 100 nM angiotensin II (AngII). HHR myocytes showed significantly higher proportions of apoptotic cells than NHR ($22.7 \pm 4.1\%$ vs $1.1 \pm 0.6\%$, $P < 0.001$). In HHR neonatal cardiomyocytes, intrinsic differences seem to predispose to significantly increased AngII-induced apoptosis. Interestingly, the bax-1/bcl-2 mRNA expression ratio was significantly higher (50%) in HHR neonatal hearts. Neonatal cardiac growth restriction, in association with increased expression of pro-apoptotic (or anti-survival) genes, is a prelude to cardiac hypertrophy at maturity in the HHR.

1. Levy D *et al.* *The New England Journal of Medicine*. 1990; **322**: 1561–1566.
2. Harrap SB *et al.* *Physiological Genomics*. 2002; **9**: 43–48.

5.1. Lessons from an elusive Conn's adenoma

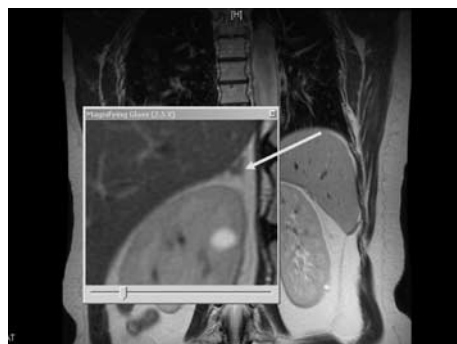
MJ Brown¹, D Appleton², NV Jamieson², D Lomas¹ and G Hoffman¹

¹University of Cambridge, Cambridge, UK and ²Addenbrooke's NHS Trust, Cambridge, UK

A 47-year old woman was found to have hypertension in 1999. In 2003, she was referred for further management, having become poorly controlled despite addition of multiple drugs. Investigations showed Na^+ 144, K^+ 3.1, Creatinine 68, renin < 2 mU/l (4–78), aldosterone 365 pmol/l (100–400), 24 h Na^+ 124 mmol. A CT scan of the adrenals was normal. Despite a trial of every known class of antihypertensive agents in combination, 24 ABPM was 220/124 mm Hg. In 2005 she was admitted for 10 days, during which treatment failed to influence her BP. Because of the low K^+ and persistently suppressed renin despite multiple drugs expected to increase renin, further investigations for Conn's were performed. Selective adrenal vein sampling revealed an aldosterone/cortisol ratio of 2.5 greater on the right than left. An MRI scan of the adrenals was negative. A repeat adrenal vein sampling was performed. The results (Table) showed a 10-fold excess on the right. Clinical review of the PACS distributed MRI pictures suggested a 3 mm nodule on one coronal section (Figure 1). She was referred for laparoscopic adrenalectomy. Pre-operatively her BP remained elevated at 220/100 despite high-dose spironolactone and amiloride. On anaesthesia, her BP fell to 70/0. The right adrenal contained a 3 mm

nodule. Within 3 days her plasma Na^+ fell to 119, and within 5 days BP was 130/70 mm Hg. Six weeks later, on amlodipine alone, BP was 140/86 mm Hg.

Vein	Aldosterone (pmol/L)	Cortisol (nmol/L)	Ratio
Right adrenal	7520	644	11.67
Left adrenal	3520	3440	1.02
IVC	254	187	1.35



Histology of the adrenal was consistent with a Conn's adenoma. There were no spironolactone bodies. This case has numerous lessons: to have high index of suspicion in patients with low-renin hypertension and hypokalaemia; to repeat investigations when the answers do not all fit; that plasma

aldosterone can remain normal in Conn's especially when the K^+ is reduced, and/or treatment includes a calcium blocker; that the coronal views on CT/MRI scanning may be more informative than axial; that a 'stress' component is consistent with, rather than excluding, a Na^+ dependent cause of hypertension.

5.2. Importance of salt in blood pressure regulation in children: a meta-analysis of controlled trials

FJ He and GA MacGregor

St George's University of London, London, UK

To assess the effect of reducing salt intake on blood pressure (BP) in children, we carried out a meta-analysis of controlled trials.

Trials were included if participants were children (≤ 18 years) and duration of salt reduction must have been for 2 or more weeks. Mean effect size was calculated using fixed effect model. As several different methods were used to assess the compliance with different salt intakes, e.g. 24-h urinary sodium, overnight urinary sodium, spot urinary sodium/creatinine ratio, food diary, we therefore calculated the percentage change in these measurements as an index of the change in salt intake.

Children and adolescents: Ten trials with 966 participants were included (Median age: 13 years, ranging from 8 to 16 years; Median duration: 4 weeks, ranging from 2 weeks to 3 years). Salt intake was reduced by 42% (IQR: 7 to 58%). There were

significant reductions in BP: systolic: -1.17 mm Hg (95% CI: -1.78 to -0.56), $P < 0.001$; diastolic: -1.29 mm Hg (-1.94 to -0.65), $P < 0.0001$.

Infants: Three trials with 551 infants were included (Median duration: 20 weeks, ranging from 8 weeks to 6 months). Salt intake was reduced by 54% (IQR: 51% to 79%). There was a significant reduction in systolic BP: -2.47 mm Hg (95% CI: -4.00 to -0.94), $P < 0.01$.

This is the first meta-analysis of salt reduction in children and demonstrates that a modest reduction in salt intake causes immediate falls in BP and, if continued, may well lessen the subsequent rise in BP with age. This would result in major reductions in cardiovascular disease. These results in conjunction with other evidence provide strong support for a reduction in salt intake in children.

5.3. The metabolic syndrome, independent of its components and other variables, is a predictor of new onset diabetes

AK Gupta¹, NR Poulter¹, B Dahlof², J Marro¹, P Sever¹ and H Wedel³

¹ICCH, NHLI, Imperial College, London, UK; ²Department of Medicine, Sahlgrenska University Hospital, Ostra, Sweden and ³Nordic School of Public Health, Goteborg, Sweden

Background: The Metabolic syndrome (MS) is strongly associated with new onset diabetes (NOD). However, whether the syndrome adds value beyond its individual components is controversial.

Objective: To evaluate whether the risk of NOD in hypertensive patients with MS is greater than that predicted by its individual components.

Methods: Among 19257 patients in ASCOT-BPLA, 14120 (mean age 62.8 years) were non-diabetic at baseline. Of these, 1366 (9.7%) subsequently developed NOD during median follow-up of 5.5 years. Three separate Cox proportional regression models were developed to assess the influence of ethnicity, age, sex, individual components of MS (using modified ATP III criteria; replacing WHR with $BMI > 30$), the syndrome itself and other baseline determinants on predictability of NOD.

Results: 4490 patients had MS at the baseline. Of these, 793 (17.7%) developed NOD (incidence rate 35.9 per 1000 pyr) as compared to 563 (5.95%) of those without MS (incidence rate 11.2 per 1000 pyr). MS at baseline was associated with more than three-fold increase in risk of NOD (Model 1) (see Table), which remained significantly high after adjusting for its components viz. fasting plasma glucose (FPG), BMI, HDLc, SBP and triglyceride (Model 2). When the other independent determinants of NOD were added to the model, MS was still found to be associated with a 23% excess risk of NOD (Model 3).

Conclusions: MS is an independent predictor of NOD even after adjusting for the risk associated with its individual components

Table Risk of NOD in hypertensive patients with MS based on Cox proportional hazard regression models

Model	Variables	Hazard ratio (95% CI)	P-value
Crude	Univariate : MS	3.18 (2.86–3.54)	$P < 0.001$
Model 1	MS+Ethnicity, age, sex	3.16 (2.83–3.52)	$P < 0.001$
Model 2	Model-1+FPG, Triglyceride, BMI, SBP, HDLc	1.20 (1.02–1.41)	$P = 0.02$
Model 3	Model-2+treatment allocation, total cholesterol, alcohol intake & use of non-cardiovascular concomitant medication	1.23 (1.04–1.44)	$P = 0.01$

5.4. Association of *GSTM* gene polymorphisms with hypertension

C Delles¹, AC Braga Marcano², PB Munroe², S Padmanabhan¹, JD McClure¹, NJ Brain¹, MJ Brown³, NJ Samani⁴, D Clayton⁵, M Farrall⁶, J Webster⁷, JMC Connell¹, MJ Caulfield² and AF Dominiczak¹

¹BHF Glasgow Cardiovascular Research Centre, University of Glasgow, Glasgow, UK; ²William Harvey Research Institute, Barts & The London Medical & Dental School, London, UK; ³Clinical Pharmacology Unit, University of Cambridge, Cambridge, UK; ⁴Department of Cardiovascular Sciences, University of Leicester, Leicester, UK; ⁵Medical Genetics, University of Cambridge, Cambridge, UK; ⁶Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, UK and ⁷Medicine and Therapeutics, Aberdeen Royal Infirmary, Aberdeen, UK

Background: Mu type glutathione-S-transferases (GSTMs) are involved in cellular defences against oxidative stress. The human *GSTM* gene cluster consists of 5 genes, *GSTM4*, 2, 1, 5 and 3. We have shown that the rat orthologue, *Gstm1*, is a positional and functional candidate gene for rodent hypertension. The aim of the current study was to test the hypothesis that this discovery can be translated to man.

Methods: We have selected 10 SNPs across the *GSTM* gene cluster and genotyped 1151 pedigrees (3453 individuals) from the MRC BRIGHT Study for these SNPs and for the common *GSTM1* deletion genotype. Analysis was performed by Transmission Disequilibrium Test (TDT) using FBAT Software.

Results: We detected highly significant association between a SNP in the 3' region of *GSTM5* (rs11807)

and hypertension ($z = 2.698$, $P = 0.007$) and significant association for two more SNPs within the *GSTM5/3* linkage disequilibrium block (rs11101992: $z = 2.443$, $P = 0.015$; rs3814309: $z = 2.215$, $P = 0.027$). The *GSTM1* deletion was also overtransmitted to affected offspring ($z = 2.339$, $P = 0.019$).

Conclusions: The *GSTM1* deletion genotype and three SNPs in the *GSTM5/3* LD block are associated with hypertension as a qualitative trait. *GSTMs*, and in particular *GSTM1*, 5 and 3, are robust candidate genes for human essential hypertension. Genetic variants in *GSTMs* may lead to reduced defences against oxidative stress and thereby to endothelial dysfunction and progression of cardiovascular disease.

5.5. Do statins reduce blood pressure? A meta-analysis of randomised controlled trials

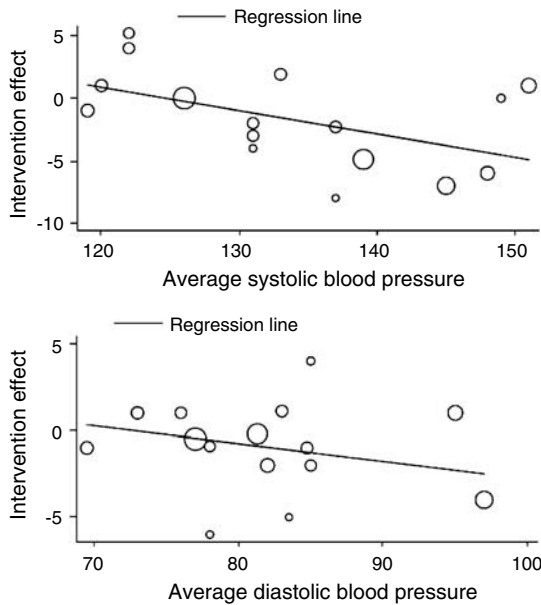
P Strazzullo¹, SM Kerry², A Barbato¹, M Versiero¹, L D'Elia¹ and FP Cappuccio³

¹Federico II University, Naples, Italy; ²St George's, London, UK and ³Warwick Medical School, Coventry, UK

Background: Very few studies have investigated the possible blood-pressure (BP) lowering effect of statins in randomised trials where BP was not being modified.

Methods and Results: We carried out a meta-analysis of the effect of statins on BP including all randomised controlled studies of statin therapy (20 trials including 887 patients) in which anti-hypertensive treatment (if any) remained unchanged throughout. Three-hundred-twenty-four and 301 patients were given a statin or placebo, respectively,

in parallel-groups trials, while 262 took part in cross-over trials receiving a statin and placebo (or probucol, in one trial). SBP was lower in patients on a statin than on placebo or control (mean difference -1.9 mm Hg, 95% CI = -3.8 to -0.1). In two trials using MBP, there was a fall of 3.1 mm Hg (CI = -14.9 to 8.6). DBP was 0.9 mm Hg lower (CI = -2.0 to 0.2) in patients receiving statin therapy. The higher the baseline BP, the greater the effect of statins on BP ($P = 0.066$ for SBP and $P = 0.023$ for DBP)(Figure). The effect was greater when the analysis was



restricted to studies with a baseline SBP > 130 mm Hg (mean Δ SBP = -4.0, CI = -5.8 to -2.2 mm Hg) or with a DBP > 80 mm Hg (mean Δ DBP = -1.2, CI = -2.6 to 0.1 mm Hg).

Conclusion: Statin therapy has a small but significant favourable effect on BP.

5.6. Functional relationship between oxidative stress and vascular stiffness in humans

C Delles¹, LU Zimmerli¹, DJ McGrane¹, AJ McKay², VL Pathi³, HJ Dargie⁴, CA Hamilton¹ and AF Dominiczak¹

¹BHF Glasgow Cardiovascular Research Centre, University of Glasgow, Glasgow, UK; ²Department of Vascular Surgery, Gartnavel General Hospital, Glasgow, UK; ³Department of Cardiothoracic Surgery, Western Infirmary, Glasgow, UK and ⁴Glasgow Cardiac Magnetic Resonance Unit, University of Glasgow, Glasgow, UK

Background: Oxidative stress is critically involved in the pathogenesis of cardiovascular disease by promoting endothelial dysfunction and reducing arterial compliance. However, a direct link between oxidative stress and vascular stiffness has not yet been demonstrated in humans.

Methods: We examined 73 patients (age: 60 ± 11 years). Of these, 49 patients had severe coronary artery disease (CAD) and underwent coronary artery bypass grafting, and 24 patients underwent surgery for removal of varicose veins but were otherwise healthy. Vascular superoxide (SO) production was examined by lucigenin-enhanced chemiluminescence in saphenous vein. Reduced to oxidised glutathione ratio (GSH/GSSG; whole blood) was measured as circulating marker of oxidative stress in a subset of patients ($n=58$). Compliance of the ascending aorta was measured in 39 patients (22 CAD and 17 control patients) by magnetic resonance

imaging (MRI) from the change in volume of the aortic segment during the cardiac cycle divided by pulse pressure.

Results: Vascular SO generation (1.00 ± 0.45 vs 0.76 ± 0.43 nmol/mg/min; $P=0.034$) and GSH/GSSG (555 ± 352 vs 824 ± 384 ; $P=0.005$) were significantly different between CAD and control patients. GSH/GSSG was not correlated with vascular SO generation ($r=-0.091$, $P=0.496$). Age ($\beta=-0.701$; $P<0.001$) and vascular SO generation ($\beta=-0.233$; $P=0.045$) were independent determinants of aortic compliance, whereas sex, body mass index, systolic blood pressure and the atherogenic index did not enter the final model (adjusted $R^2=0.553$).

Conclusions: We were able to prove the relationship between oxidative stress and arteriosclerosis by measuring SO generation directly in the vessel wall and using state-of-the-art MRI-based imaging to assess vascular stiffness.

7.4. Cardiovascular event reduction in the Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT): combined benefits of blood pressure reduction, lipid-lowering and treatment with a 'newer' compared with an 'older' antihypertensive treatment regimen

P Sever¹, N Poulter¹, B Dahlöf² and H Wedel³

¹Imperial College London, London, UK; ²University of Goteborg, Goteborg, Sweden and ³Nordiska School of Public Health, Goteborg, Sweden

ASCOT recruited hypertensive subjects who were poorly controlled (mean BP 164/95 mm Hg). The trial design allowed an independent comparison on cardiovascular outcome of the effects of atorvastatin and placebo, and of a 'newer' treatment regimen of a calcium channel blocker (amlodipine) adding an angiotensin converting enzyme inhibitor (perindopril) as required compared with an 'older' regimen of a beta-blocker (atenolol) adding a thiazide diuretic (bendroflumethiazide-K) as required; ASCOT-BPLA. In addition, the impact of good blood pressure control (to an average of 138/80 mm Hg by the end of ASCOT-LLA) could be assessed.

Event rates during the first 6 months of the trial for the combined BP treatment limbs among those not receiving a statin were 14.1/1000 patient-years for non-fatal myocardial infarction and fatal coronary

heart disease (CHD), and 11.4/1000 patient-years for stroke. These event rates fell to 9.1/1000 patient-years and 6.8/1000 patient-years respectively by the end of ASCOT-LLA. These benefits attributable to BP control represent a 35% reduction in CHD events and a 40% reduction in stroke.

In ASCOT-LLA, compared with placebo, atorvastatin reduced the incidence of CHD events by 36% and stroke by 27%. In ASCOT-BPLA, the benefits of the newer regimen on CHD and stroke events were 10 and 23% respectively. Combining these effects with those of good BP control, the estimated reduction in both CHD and stroke outcomes is approximately 65%.

The importance of good BP control together with optimal therapeutic intervention on BP and lipid-lowering confers extensive advantages to the hypertensive patient.

7.5. Continued benefit on cardiovascular outcomes in the Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT) two years after closure of the lipid-lowering arm (LLA)

P Sever¹, N Poulter¹, B Dahlöf² and H Wedel³

¹Imperial College London, London, UK; ²University of Goteborg, Goteborg, Sweden and ³Nordiska School of Public Health, Goteborg, Sweden

Background: Reductions in coronary heart disease (CHD) and stroke events have been demonstrated with atorvastatin in hypertensive subjects with normal or moderately raised cholesterol levels in ASCOT-LLA.

Methods: In ASCOT-LLA 10,305 patients with total cholesterol levels ≤ 6.5 mmol/l were randomised to either atorvastatin (10 mg) or placebo. The study was stopped prematurely after a follow-up period of 3.3 years. Patients, however, continued in the blood pressure lowering arm (BPLA) of the trial for a further two years. Following closure of ASCOT-LLA, patients originally assigned placebo were offered atorvastatin, and of those originally assigned atorvastatin, many chose to discontinue the drug.

Results: Total and LDL-cholesterol levels, which at the end of the LLA were approximately 1 mmol/l lower in those receiving atorvastatin by the end of BPLA were identical in both those originally assigned atorvastatin and placebo.

At the closure of LLA, there were significant risk reductions in non-fatal myocardial infarction plus fatal CHD (HR 0.64, CI 0.50–0.83, $P=0.0005$), total cardiovascular events and procedures (HR 0.75, CI 0.69–0.90, $P=0.0005$) and stroke (HR 0.73, CI 0.56–0.96, $P=0.024$).

However, despite equalisation of lipid profiles in the two treatment limbs over the ensuing two years, relative risk reductions in favour of those originally assigned atorvastatin, remained unchanged after an average 5.5 years follow-up. For non-fatal myocardial infarction plus fatal CHD (HR 0.64, CI 0.53–0.78, $P<0.0001$), total cardiovascular events and procedures (HR 0.81, CI 0.73–0.89, $P<0.0001$) and stroke (HR 0.77, CI 0.63–0.95, $P=0.013$).

Conclusion: Persistent benefits have been demonstrated in those initially assigned lipid-lowering therapy in ASCOT, despite the achievement of similar lipid profiles by the end of the trial, additional explanations are required to account for these observations.

PA.1. Microarray profiling identifies genes and pathways contributing to the development of hypertension

MW McBride, JD McClure, JM Polke, D Graham and AF Dominiczak

BHF Glasgow Cardiovascular Research Centre, University of Glasgow, Glasgow, UK

Objectives: The aims of our study were to utilise congenic strains and microarray profiling during the development of hypertension to identify genes and pathways underlying hypertension in the SHRSP_{Gla}.

Methods: We compared 5 and 16 week old renal RNA from the SHRSP_{Gla}, WKY_{Gla} and congenic strain SP.WKY_{Gla}2c (2c) that harbours *Gstm1*, a previously identified positional candidate gene for hypertension. Microarray expression profiling used the RG U34 A, B and C Gene Chips ($n=3$). Differential expression was determined by the ranked products method with a false discovery rate (FDR) $P<0.05$ and fold change (FC) >2 . Disrupted pathways were identified (Ingenuity Pathway Analysis).

Results: 120 canonical pathways, representing more than 6500 genes and interactions, were compared at 5 and 16 weeks of age with 9 and 2 pathways

significantly disrupted respectively ($P<0.05$). Multiple genes were differentially expressed in glutathione metabolism localizing to the congenic interval including *Gstm1* (2c vs SHRSP_{Gla}, FDR = 0.00, FC = 9.4, WKY_{Gla} vs SHRSP_{Gla} FDR = 0.00, FC = 7.5) and solute carrier 7 (*Slc7a12*) (2c vs SHRSP_{Gla}, FDR = 0.00 FC = -2.9, WKY_{Gla} vs SHRSP_{Gla} FDR = 0.00, FC = -2.3) and gamma-glutamyltransferase 1 (*Ggt1*) (2c vs SHRSP_{Gla}, FDR = 0.03 FC = 2.1, WKY_{Gla} vs SHRSP_{Gla}, FDR = 0.03, FC = 2.2) which does not localise to the congenic interval.

Conclusions: Pathway analysis highlights multiple disruptions to glutathione metabolism implicating positional genes within the congenic interval and, for the first time, implicates genes that may be under transcriptional control of the congenic segment.

PA.2. Candidate gene identification for cardiac hypertrophy on rat chromosome 3

D Graham, K Gilday, MW McBride and AF Dominiczak

BHF Glasgow Cardiovascular Research Centre, Glasgow, UK

Objectives: Linkage studies in Dahl salt-sensitive and spontaneously hypertensive rat strains have previously identified overlapping QTL for pulse pressure, cardiac and left ventricular mass mapping to rat chromosome 3. Using an improved chromosome 3 genetic map this study aimed to investigate cardiac mass QTL in the SHRSP_{Gla} strain and identify candidate genes based on comparative mapping.

Methods: 140 F₂ rats (SHRSP x WKY) were sacrificed at 21 weeks of age after 3-week NaCl challenge (1%). Cardiac and left ventricular mass, were estimated from heart, left ventricle plus septum and body weight measurements. Linkage analysis was carried out using MapManager QTX. Regions of synteny were identified using Ensembl. RNA expression from hearts of 6 and 21-week old SHRSP and WKY were analysed using quantitative RT-PCR.

Results: Linkage analysis confirmed significant pulse pressure QTL (maximal LOD score 4.9,

$P=0.00001$, at marker D3Rat50) and identified significant cardiac mass QTL (maximal LOD score 3.0, $P=0.0016$ at marker D3Mgh16) on rat chromosome 3. Conserved genome analysis identified retinoid X receptor α (RXR α) as a positional candidate gene with syntenic regions on mouse chromosome 2 and human chromosome 9. RXR α expression was significantly increased in SHRSP hearts at 6 weeks ($P=0.0023$, $F=95.1$) and 21 ($P=0.05$, $F=7.3$) weeks compared to WKY. Salt loading significantly reduced RXR α expression in 21-week SHRSP hearts to WKY levels ($P=0.002$, $F=23.3$).

Conclusion: We have confirmed multiple overlapping cardiovascular QTL on rat chromosome 3 and identified the positional candidate RXR α with potential contribution to both hypertensive and hypertrophic phenotypes in the SHRSP_{Gla}.

PA.3. Interactions between multiple promoter polymorphisms are required for reduced expression of GSTM1 in the SHRSP

JM Polke, MW McBride, SA Nicklin, AH Baker, D Graham and AF Dominiczak

BHF Glasgow Cardiovascular Research Centre, Glasgow, UK

Objectives: Glutathione s-transferase mu type 1 (*Gstm1*) is a positional and functional hypertension candidate gene in the stroke-prone spontaneously hypertensive rat (SHRSP_{Gla}). This study examined the expression of *Gstm1* in a panel of normotensive and hypertensive strains and aimed to identify promoter single-nucleotide polymorphisms (SNPs) responsible for reduced SHRSP_{Gla} *Gstm1* expression. **Methods:** qRT-PCR measured renal *Gstm1* expression in the SHRSP_{Gla}, WKY_{Gla}, Brown Norway (BN) and spontaneously hypertensive rat (SHR) strains. 2.5 kb of the *Gstm1* promoter was sequenced in each strain. SHRSP_{Gla} and WKY_{Gla} *Gstm1* promoters from 0.9 kb to 2.5 kb covering increasing SNPs were cloned into the pGL3-Basic luciferase plasmid. These were cotransfected in triplicate into a rat kidney epithelial cell line (NRK52E) with pMV10 beta-galactosidase plasmid. Experiments were repeated three times.

Results: *Gstm1* expression was downregulated 4-fold in the SHRSP and SHR compared to the WKY and BN; reduced expression correlated with 13 promoter polymorphisms not found in the WKY and BN. There was a consistent significant 2.3–2.6 fold ($P < 0.015$) reduction in luciferase activity from the SHRSP_{Gla} 1.6 kb construct compared to the WKY_{Gla} 1.6 kb construct. There were no significant differences between other constructs (0.9 kb/7SNPs; 2.2 kb/12SNPs; 2.5 kb/13SNPs). This implicated 5 SHRSP_{Gla} variants which were cloned on to the WKY_{Gla} 1.6 kb background, the remaining 7 1.6 kb SHRSP_{Gla} SNPs were also cloned; the resultant luciferase activities of these plasmids were not significantly different to WKY_{Gla} 1.6 kb.

Conclusions: These results demonstrate reduced *Gstm1* expression correlating with hypertension in multiple rat strains and a complex interaction of functional sequence elements operating in the *Gstm1* promoter.

PA.4. Matrix metalloproteinase-9 polymorphism contributes to large artery stiffness in essential hypertension

S Zhou, J Feely, P Spiers and A Mahmud

Department of Pharmacology & Therapeutics, Trinity College and Hypertension Clinic, St James's Hospital, Dublin, Ireland

Matrix metalloproteinases (MMP)-9 is an important member of the matrix metalloproteinase family. A cytosine (C)-thymidine (T) single nucleotide polymorphism (SNP) at position -1562 in the MMP-9 promoter is reported to affect expression of this gene and the CT and TT genotypes have high activity of the MMP-9 gene promoter, increasing the risk for cardiovascular disease. In this study we investigated the association between the MMP-9 polymorphism and arterial stiffness in essential hypertension. We studied 182 untreated hypertensive patients mean age 48 ± 1 years (96 male). Genomic DNA of the patients was amplified by polymerase chain reaction (PCR) and analysed by restriction endonuclease digestion. Arterial stiffness was measured by carotid-femoral pulse wave velocity (PWV) and aortic wave reflection by augmentation index (AIx). The

frequencies of polymorphic genotypes in the promoter of MMP-9 were CC 70.3%, CT 26.4%, and TT 3.3%. Compared with CC homozygotes, T allele carriers (CT and TT) had higher PWV (11.0 ± 0.2 vs 10 ± 0.1 , $P < 0.05$), mean arterial pressure (118 ± 1 vs 112 ± 1 , $P < 0.01$) and diastolic pressure (95 ± 1 vs 90 ± 1 , $P < 0.01$) but similar AIx. As MMP-9 degrades the arterial extracellular matrix, it is likely that the genetic alteration of MMP-9 expression affects arterial structure and therefore arterial stiffness in hypertension. Our results indicate that the CT and TT genotype in the MMP-9 promoter may have higher activity to degrade the arterial elastic matrix and this may be associated with increased arterial stiffness in large but not small muscular arteries in essential hypertension.

PA.5. Adiponectin gene polymorphism (G276 T) contributes to arterial stiffness in essential hypertension

S Zhou, J Feely, P Jerrard-Dunne, P Spiers and A Mahmud

Department of Pharmacology & Therapeutics, Trinity College and Hypertension Clinic, St James's Hospital, Dublin, Ireland

Adiponectin, the anti-inflammatory adipocytokine is reduced in hypertension, diabetes and coronary artery disease (CAD). We have previously shown that plasma adiponectin levels are related to arterial stiffness in hypertension¹. In the adiponectin gene, G to T substitution at position 276 in intron 2 (SNP276G>T) is associated with type 2 diabetes and insulin resistance. However, whether this polymorphism may contribute to arterial stiffness is not known. We studied untreated patients with hypertension ($n = 221$, 109 Female). G>276 T was determined by Restriction Fragment Length Polymorphism. Arterial stiffness was measured as pulse wave velocity (PWV) (Complior) and augmentation index (AIx). Data analysed using JMP (SAS for Windows) expressed as mean \pm s.e.m., $P < 0.05$ considered significant. Results are given in the Table. The frequencies of polymorphic genotypes were G/G 53%, G/T 37%, and T/T 10%. The PWV was significantly higher in the G allele carriers and this remained significant after adjusting for age, BP and

	T/T homozygotes	G/G and G/T	P value
Age (years)	43.41 \pm 3.13	49.10 \pm 0.88	$P = 0.09$
Systolic BP (mmHg)	146.32 \pm 4.53	154.81 \pm 1.37	$P = 0.043$
Diastolic BP (mmHg)	85.73 \pm 2.48	91.08 \pm 0.77	$P = 0.06$
PWV (m/sec)	8.87 \pm 0.38	10.17 \pm 0.15	$P = 0.005$
AIx (%)	24.82 \pm 3.41	28.76 \pm 0.92	$P = 0.32$
Glucose level (mmol/l)	4.67 \pm 0.20	5.62 \pm 0.13	$P = 0.0004$

gender ($P < 0.05$). There was no significant difference in cholesterol, high-density lipoprotein or triglycerides between the two groups.

The SNP276 of the adiponectin gene contributes to stiffness in the large elastic arteries and impaired glucose tolerance in patients with essential hypertension.

1. Mahmud A, Feely J. Adiponectin and arterial stiffness. *Am J Hypertens.* 2005 Dec; **18**: 1543–1548.

PB.1. Advanced glycation end products and arterial stiffness in hypertension

A Mahmud, M McNulty and J Feely

Department of Pharmacology & Therapeutics, Trinity College & Hypertension Clinic, St James's Hospital, Dublin, Ireland

The formation of advanced glycation end-products (AGEs) is associated with arterial stiffness in experimental models and an AGEs cross-link breaker, alagebrium (formerly ALT-711), has been shown to reduce arterial stiffness in elderly subjects. We related plasma concentrations of advanced AGEs, measured using a non-competitive immunoassay, and markers of aortic stiffness- pulse wave velocity (PWV) and augmentation index (AIx), an index of aortic wave reflection, in 46 subjects mean age 47 ± 2 years, comprising of 30 untreated hypertensive and 16 normotensive subjects. Results were analysed using univariate and multiple logistic regression analysis.

Plasma AGEs were significantly higher in hypertensive than normotensive subjects (7.8 ± 1 versus

3 ± 1 uAGE/ml; $P < 0.0001$). There was a significant relationship between plasma AGEs and aortic PWV ($r = 0.49$, $P < 0.01$) but not with AIx. In a stepwise regression model age, total cholesterol, plasma AGEs and smoking status explained 67% of the variability in PWV. For AIx, the only variables that entered the model were age, gender and heart rate ($R^2 = 0.38$, $P < 0.0001$) with no contribution from plasma AGEs. Plasma AGEs concentrations are significantly higher in hypertensive versus normotensive subjects and are related to aortic stiffness independent of age and blood pressure, with no relationship with aortic wave reflection. Plasma AGEs may play a blood pressure independent role in large vessel but not small vessel remodelling in essential hypertension.

PB.2. Wave reflection and arterial stiffness are related to left ventricular diastolic dysfunction independently of left ventricular geometry in essential hypertension

A Mahmud¹, I Al Muntasir², G King², P Crean² and J Feely¹

¹Department of Pharmacology & Therapeutics, Trinity College & Hypertension Clinic, St James's Hospital, Dublin, Ireland and ²Department of Cardiology, St James's Hospital, Dublin, Ireland

Arterial stiffness is an independent predictor of cardiovascular and all-cause mortality and morbidity. Ventricular stiffness, as evidenced by incomplete relaxation of the ventricle during early diastolic filling, is also associated with increasing age and high blood pressure. We studied 104 consecutive (mean age 47 ± 1 , mean \pm s.e.m.) white healthy hypertensive patients based on 3 outpatient measures of blood pressure (BP) $>140/90$ mm Hg and confirmed by ambulatory BP ($>135/80$ mm Hg). Brachial BP was measured using an automated oscillometric technique (Omron), pulse wave velocity (PWV) with the Complior and augmentation index (AIx) with the SphygmoCor. The echocardiographic assessment involved both standard M-Mode and Doppler Tissue Imaging (DTI). The peak early diastolic velocity (Ea) and the peak atrial systolic

velocity (Aa) were recorded at multiple sites and averaged and the Ea/Aa calculated. Ventricular relative wall thickness (RWT) was defined as the sum of septal and posterior wall thickness divided by end-diastolic dimension. There was a significant and inverse relationship between Ea/Aa and AIx ($r = -0.49$, $P < 0.0001$) and PWV ($r = -0.50$, $P < 0.0001$). The AIx and PWV were higher in patients with diastolic dysfunction (Ea/Aa < 1.0). The AIx, but not PWV, was an independent determinant of Ea/Aa ($r^2 = 0.44$, $P < 0.0001$) along with age, heart rate and RWT. Thus the arteries and the ventricle may share a common pathway whereby structural alterations occur in both in parallel. On the other hand, a stiff vasculature may precede the development of left ventricular dysfunction and identified early enough, may even be prevented.

PB.3. Isolated systolic hypertension is characterised by increased aortic stiffness and endothelial dysfunction

S Wallace¹, Y Yasmin¹, K Maki-Petaja¹, A Booth¹, C McEniery¹, J Brown² and I Wilkinson¹

¹Clinical Pharmacology, University of Cambridge, Cambridge, UK and ²Trinity College, University of Cambridge, Cambridge, UK

Objective: Isolated systolic hypertension (ISH) is associated with increased cardiovascular risk, and results from large artery stiffening. Large artery stiffness is determined by structural components within the vasculature but also by functional factors including nitric oxide and ET-1.

We hypothesized that endothelial dysfunction may account for the increased arterial stiffness in patients with ISH. The aim of this study was to investigate the relationship between endothelial function and arterial stiffness in patients with ISH and controls.

Methods: We studied 98 patients; 23 ISH patients, 26 age-matched controls and 49 young controls. Aortic PWV was derived using sequential carotid/femoral waveform recordings (SphygmoCor). To assess conduit artery endothelial function we used the technique of flow-mediated dilatation (FMD). This was then followed by administration of

glyceryl trinitrate to assess independent endothelial function.

Results: Aortic PWV was higher in patients with ISH compared with age-matched controls (10.88 vs 8.86 m/s; $P < 0.0001$). FMD was reduced in patients with ISH (2.7 vs 4.9%). Aortic pulse wave analysis was also higher and FMD lower in older versus young controls (6.51 vs 8.86 m/s, $P < 0.0001$ and 4.9 vs 6.7%; $P < 0.0001$ for both). The GTN responses did not differ. Overall aortic PWV correlated with FMD ($R = 0.4$, $P = < 0.0001$) which remained significant after adjustment for mean arterial pressure, age, heart rate, cholesterol and glucose ($P = 0.02$).

Conclusions: ISH patients have higher aortic PWV and decreased endothelial function compared with age-matched controls. Our results suggest that endothelial function contributes significantly to increased arterial stiffness in patients with ISH and with age.

PB.4. Association between large and small artery function in relation to glycaemia and blood pressure in postnatal women

M Banerjee, RA Malik, AM Heagerty and JK Cruickshank

Manchester University, Manchester, UK

Introduction: Diabetes and hypertension affect large and small artery function. Aortic pulse wave velocity (aPWV) and laser Doppler fluximetry (LDF) are well established measures of aortic and microcirculatory function. We investigated the relationship between aPWV and LDF in a group of postnatal women who had undergone an oral glucose tolerance test (OGTT) during pregnancy.

Patients and Methods: 142 postnatal women (age-36.2 years 95% CI 35.1–37.2) had standardised measures of aPWV, LDF (over the foot, before and after thermal stimulation) and blood biochemistry.

Results: The aPWV was inversely correlated with basal LDF ($r = -0.25$, $P = 0.018$) and directly with systolic blood pressure ($r = -0.18$, $P = 0.036$). Heat

augmented LDF was related to 2 h glucose ($r = -0.54$, $P < 0.001$). Comparing women who had gestational diabetes (GDM, $n = 17$) with those who did not, aPWV was similar but thermally augmented LDF was lower at (mean, 95% CI) 123, 64–183% versus 192, 150–233% respectively ($P = 0.05$). On multiple regression analysis, adjusting for age, fasting glucose, body mass index and smoking history, diastolic blood pressure was directly related to aPWV ($\beta = 0.72$, $P = 0.03$) while age influenced the basal LDF result ($\beta = 0.3$, $P = 0.04$).

Conclusion: Large and small vessel functions are closely related and are modulated by glycaemia and blood pressure, even in women without overt diabetes or hypertension.

PB.5. Circadian blood pressure variation: relationship between non-dipper status and measures of arterial stiffness

P Jerrard-Dunne, A Mahmud and J Feely

Department of Pharmacology & Therapeutics, Trinity Centre for Health Sciences, St James's Hospital, Dublin, Ireland

Compared with dippers, hypertensive individuals with a non-dipping nocturnal BP profile have more target organ damage and a worse cardiovascular prognosis possibly mediated through increased arterial stiffness. This study aimed to examine arterial stiffness and dipping patterns in a population of 341 untreated hypertensive subjects, mean age 48 ± 8 , 55% male. Dipping was defined as a 10–20% fall in nocturnal BP; extreme-dipping as $> 20\%$; non-dipping as $< 10\%$ and reverse-dipping as $\leq 0\%$ fall in nocturnal BP, using a patient diary to determine sleep-time. Aortic pulse wave velocity (PWV) (Complior) and augmentation index (AIx) (Sphygmocor) were measured. The groups did not differ by age, gender, 24 h or daytime mean BP, BMI, smoking, cholesterol, glucose, renin or aldosterone. The relationship between PWV and dipper-status was J-shaped, with extreme-dippers and reverse-

dippers having the highest PWV. Non-dippers and reverse-dippers had significantly higher PWV (mean (s.d.) 10.9 (2.5) m/s and 11.5 (3.8) m/s respectively) when compared with dippers (9.8 (1.8) m/s). Following multivariate adjustment for age, gender, BP, heart rate and smoking, reverse-dippers had significantly higher PWV than either dippers or non-dippers ($P = 0.005$ and $P = 0.031$ respectively). Dipper status was not associated with AIx. The presence of a reverse-dipper pattern on ambulatory blood-pressure monitoring identifies a population group with increased PWV. This difference could not be explained by the measured risk factors. Prospective interventional studies would be required to determine whether this is a cause or effect relationship and whether eliminating the non-dipper effect improves arterial stiffness and consequently reduces cardiovascular morbidity.

PC.1. The polypill for hypertension – greater reduction in blood pressure compared to monotherapy

A Mahmud and J Feely

Department of Pharmacology & Therapeutics, Trinity College and Hypertension Clinic, St James's Hospital, Dublin, Ireland

To determine whether a 'polypill' containing a quarter of the standard dose of four antihypertensive agents has greater efficacy than standard dose of each individually, we randomised in a prospective fashion, 108 untreated Caucasian hypertensive patients [55% male] aged 50 ± 1 year (mean \pm s.e.m.), with mean clinic blood pressure (BP) $160 \pm 1/96 \pm 1$ mm Hg to amlodipine (5 mg, $n=22$), atenolol (50 mg, $n=20$), bendroflumethiazide (2.5 mg, $n=21$), captopril (50 mg twice daily, $n=22$) or a capsule containing each of the four above at one quarter dosage ($n=22$) in a parallel group design for 4 weeks. Blood pressure was measured using a semi-automated device (Omron 705). The reduction in mean arterial pressure (MAP) with the combined preparation was compared to that of the individual components. For analysis of changes in BP, percentage reductions were calculated using Oldham

correction: $\text{change in BP} = \frac{\text{Pre-treatment BP} + \text{Post-treatment BP}}{2}$. Statistical analysis was performed with JMP Version 5.0 (SAS for Windows) using ANOVA and Tukey-Kramer HSD. The reduction in MAP with the combination [$16\% \pm 2$] was significantly greater than that with individual agents; amlodipine [$8\% \pm 2$, $P < 0.005$], atenolol [$9\% \pm 2$, $P < 0.005$], bendroflumethiazide [$5\% \pm 1$, $P < 0.001$], captopril [$9\% \pm 1$, $P < 0.01$]. In addition the reduction in systolic [$16\% \pm 1$, $P < 0.005$] and diastolic [$15\% \pm 2$, $P = 0.06$] BP was greater with the combination. More patients achieved a BP $< 140/90$ mm Hg with the polypill [60%] than the individual drugs [14–45%, $P < 0.05$]. A low dose combination of multiple agents representing four classes of standard antihypertensive agents was more effective than a standard single dose of each agent individually.

PC.2. Peripheral nerve function in hypertension

L Edwards, C Ring, JB Winer and U Martin

University of Birmingham, Birmingham, UK

Essential hypertension is associated with hypoalgesia. This phenomenon may be partly due to deficits in peripheral nerve function. The present study investigated the association between hypertension and peripheral nerve function, in 17 unmedicated essential hypertensives and 26 normotensives. Sensory nerve action potentials (SAPs) and compound motor action potentials (MAPs) were elicited by supramaximal electrocutaneous stimulation of the median nerve at the wrist and elbow. SAPs were recorded in the index finger at the proximal interphalangeal joint. MAPs were recorded in the abductor pollicis brevis. Sensory and motor nerve conduction velocities (m/sec) and SAP onset-to-peak amplitudes (μV) were determined from the averaged waveforms of 36 trials.

Mean daytime ambulatory blood pressures were higher ($P < 0.0001$) in hypertensives (149/96 mm Hg) compared to normotensives (118/77 mm Hg). SAP amplitudes elicited by wrist stimulation were sig-

nificantly lower ($P < 0.05$) in hypertensives ($M = 19 \mu\text{V}$), than normotensives ($M = 28 \mu\text{V}$). SAP amplitudes elicited by elbow stimulation were also significantly lower ($P < 0.05$) in hypertensives ($M = 11 \mu\text{V}$) than normotensives ($M = 15 \mu\text{V}$). These effects survived adjustment for stimulation-to-recording distance, finger circumference, gender, age and alcohol consumption. Sensory and motor nerve conduction velocities were unaffected by blood pressure status.

While sensory nerve conduction velocity was not affected by hypertension, the current data, describing lower amplitude SAPs in unmedicated essential hypertensives, suggest that the number of active nerve fibres in the median nerve may be reduced in unmedicated hypertension and, therefore, individuals with high blood pressure may suffer from mild subclinical peripheral neuropathy in sensory afferents.

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PC.3. Detection and analysis of low-renin hypertension in young patients

MJ Brown, F Baker, A Mitchell, D Picton, SJ Hood and K Taylor

University of Cambridge, Cambridge, UK

Low-renin, diuretic-responsive hypertension is uncommon at a young age. We have deliberately sought these patients since they are likely to be enriched in genetic variants contributing to hypertension. In a retrospective study, we previously found a low-renin on ACEi or ARB to be especially predictive of diuretic response (Hood *et al. Clin Med* 2005; **5**: 55–60). In a prospective study, therefore, we have measured patients' BP, renin and aldosterone before and after two weeks treatment with losartan 100 mg. Our objectives are [i] to recruit a panel of 50 patients giving us >99% power to detect any SNP contributing to at least 5% of low-renin hypertension; and [ii] to determine whether patients with low-renin hypertension due to angiotensin hypersensitivity could be separated from patients more likely to have gain-of-function mutations in a gene encoding renal Na⁺ channels, or their regulators. From 2002 to 2006, patients were recruited in three ways: [i] through establishment of a young patients clinic; [ii] by contacting offspring of older, low-renin patients; and [iii] by screening in general practice hypertensive patients aged <45 who were poorly controlled on monotherapy with an 'AB' drug, or well-controlled on a 'CD' drug. To date we have identified

41 patients aged ≤45 with plasma renin <10 mU/l (≤0.5 pmol/ml/h) despite having a 24-h Na⁺ excretion <160 mmol. The responses to losartan were variable (Table). In 18/41 BP fell to ≤140/85 mm Hg, mainly in patients whose renin was not completely suppressed. The BP response weakly correlated with both baseline renin ($r=0.38$, $P=0.05$) and the rise in renin on treatment ($r=0.41$, $P<0.05$). In these low-renin patients, there was no significant correlation between plasma renin and aldosterone, and no significant fall in aldosterone on losartan. We conclude that [i] low-renin hypertension is uncommon in young patients; [ii] the phenotype is heterogeneous and can be dissected by response to blockade of the renin system into those in whom renin is suppressed by angiotensin hypersensitivity and those who are retaining excess sodium.

	Age	BP (mmHg)	Renin (mU/L)	Aldosterone (pmol/L)
Baseline	35 ± 7	152 ± 18/97 ± 13	5.0 ± 2.8	330 ± 87
Losartan		143 ± 22/92 ± 14	31 ± 43	284 ± 123

PC.4. Do anti-oxidants cause gestational hypertension?

AL Briley, L Poston and AH Shennan

King's College London, London, UK

Background: Pre-eclampsia is associated with oxidative stress. We have previously shown that Vitamin C (1000 mg) and E (400IU) do not reduce the incidence of pre-eclampsia in women at risk (*Lancet* 2006; **367**: 1145–1154). An unexpected finding in this trial was an increase in gestational hypertension (a pre-defined secondary endpoint) in supplemented women, and need for intravenous anti-hypertensive medication. Another randomised controlled trial (RCT) using the same intervention in nulliparous women, (subsequently reported) also demonstrated a significant, and unexpected, increase in the need for any anti-hypertensive medication in the treatment arm (*NEJM* 2006; **354**: 1796–1806).

Methods: We therefore analysed data from 4 RCTs which used the same intervention in pregnancy (two

already cited, plus *Lancet* 1999; **354**: 810–816, *AmJOG* 2005; **192**: 520–521).

Results: Data from 3 of these studies gave information about gestational hypertension, and therefore were meta-analysed. The incidence of gestational hypertension was significantly increased in women receiving supplements compared to placebo: 222/2210 (10.05%) vs 172/2222 (7.74%), RR 1.67 (95% CI, 1.03–2.69).

Conclusions: High doses of antioxidants in the form of C and E increase the incidence of gestational hypertension, and associated need for anti-hypertensive medication. Further work needs to establish the relationship between oxidative stress, high dose anti-oxidants and hypertension.

PC. 5. Body size and blood pressure: a collaborative analysis of 18,072 participants from Africa and the African diaspora

FP Cappuccio¹, SM Kerry², A Adeyemo³, A Luke⁴, AGB Amoah⁵, P Bovet⁶, MD Connor⁷, T Forrester⁸, J-P Gervasoni⁹, G Kimballi Kaki¹⁰, J Plange-Rhule¹¹, M Thorogood¹ and RS Cooper⁴

¹Warwick Medical School, Coventry, UK; ²St George's Hospital, London, UK; ³University of Ibadan, Ibadan, Nigeria; ⁴Loyola University, Maywood, IL, United States; ⁵University of Ghana, Accra, Ghana; ⁶University of Lausanne & MoH, CH & Victoria, Seychelles; ⁷University of Witwatersrand, Johannesburg, South Africa; ⁸University of West Indies, Kingston, Jamaica; ⁹University of Lausanne & Regional Ministry, CH & Dar es Salam, Tanzania and ¹⁰CHU, Brazzaville, Congo, DR; ¹¹Komfo Anokye Teaching Hospital, Kumasi, Ghana

Background: Blood pressure (BP) is associated with body mass index (BMI) in populations worldwide. However, the relationship may vary in populations of African origin.

Objective: To compare the relationship between BP and BMI in the African diaspora.

Design, setting and participants: Surveys were identified from an investigative network in Africa, Caribbean, UK and US. Raw data on 18,072 individual participants (35–64 years, 44% men) for age, height, weight, BP and treatment of hypertension (Hpt) were pooled. Participation rate varied from 54 to >90%. We calculated age-specific and age-adjusted estimates of BP, BMI, Hpt prevalence and multivariate regression analysis to estimate the relationship between BP and BMI by country and by sex.

Results: BP and Hpt prevalence increased with age in each country. BMI varied by up to 50% between countries. In every country and sex-group there was a positive relationship between both SBP and DBP and BMI. The slopes for SBP varied from 0.27 mm Hg per unit BMI [95% CI –0.01 to 0.56] in US to 1.72 [0.92 to 2.53] in Kumasi in men and from 0.08 [–0.54 to 0.72] in S Africa to 1.32 [0.98 to 1.66] in the Republic of Congo in women. Similar trends were seen for DBP. The slopes were significantly shallower, the higher the mean BMI (meta-regression $\beta = -0.10$ mm Hg SBP*unit BMI; $\beta = -0.08$ mm Hg DBP*unit BMI, both $P < 0.001$).

Conclusions: BP and BMI vary substantially among the African diaspora. The effect of BMI on BP levels diminishes as BMI increases. Lean mass may play a role in the relationship between BMI and BP in these populations.

PD.1. Discovery of a biomarker pattern in urine for the diagnosis of coronary artery disease

LU Zimmerli¹, E Schiffer², A Pitt³, K Graham⁴, H Mischak², W Kolch⁵, C Delles¹ and AF Dominiczak¹

¹BHF Glasgow Cardiovascular Research Centre, University of Glasgow, Glasgow, UK; ²Mosaïques Diagnostics and Therapeutics AG, Hanover, Germany; ³Sir Henry Wellcome Functional Genomics Facility, University of Glasgow, Glasgow, UK; ⁴Physiotherapy Department, Western Infirmary, Glasgow, UK and ⁵The Beatson Institute for Cancer Research, Glasgow, UK

Objective: Proteomics is a powerful non-invasive tool for diagnosis of a variety of diseases. We tested whether signatures of urinary polypeptides can be used for the diagnosis of coronary artery disease (CAD). We also aimed to obtain sequences of potential biomarkers that determine CAD specific polypeptide pattern.

Design and method: We examined 66 subjects (mean age, 61±12 years), of whom 38 had severe CAD confirmed by coronary angiography and 28 had no evidence of CAD. Spot urine was analysed using capillary electrophoresis coupled to ESI-TOF mass spectrometry enabling characterisation of more than 1000 polypeptides per sample. Data were evaluated using MosaVisu and MosaCluster software to define a CAD-specific pattern. To identify biomarkers of the indicative polypeptide pattern, peptide sequencing was performed using tandem mass spectrometry.

Results: We identified a CAD-specific polypeptide pattern consisting of 24 potential biomarkers. This pattern showed sensitivity of 100% (95% CI, 87–100) and specificity of 96% (95% CI, 81–99) in cross validation to distinguish between CAD and controls. Within differentially regulated polypeptides we identified fibrillar collagen fragments as specific biomarkers for CAD.

Conclusions: We validated urinary proteomics as a tool to distinguish between patients with CAD and healthy controls. Identification of peptides determining the CAD-specific pattern may lead to new insights into the pathogenesis of CAD. Urinary proteomics has the potential to be used as a non-invasive tool in CAD diagnosis and will in the future be extended to monitoring of disease severity and progression.

PD.2. South Asians have narrower coronary arteries and different remodelling responses to disease compared to Europeans

T Tillin¹, H Dhutia¹, J Chambers¹, I Malik¹, E Coady¹, J Mayet¹, A Wright¹, J Kooner¹, A Shore², S Thom¹, N Chaturvedi¹ and A Hughes¹

¹International Centre for Circulatory Health, Imperial College, Hammersmith and St Mary's Hospitals, London, UK and ²Peninsula Medical School, Exeter, UK

Objectives: To compare left anterior descending (LAD) artery diameter in relation to stenosis and coronary artery calcification in British South Asian and White European men.

Methods: 41 South Asian and 42 European men (mean age 64 ± 9 years) with coronary artery disease were studied. All had similar symptoms. Vessel reference diameter and degree of stenosis were calculated using quantitative coronary angiography. Extent of atherosclerotic disease in the LAD was assessed using calcification scores (CACS) measured by multi-slice Computed Tomography. Fasting bloods and blood pressure were measured. LAD measurements were made in the proximal 2.5 cm segment.

Results: South Asian men had more LAD stenosis than European men (47 vs 32%, $P=0.033$), but CACS did not differ. South Asian men with CACS in

the lowest tertile (0–22 HU), had significantly narrower LAD diameters than Europeans (2.8 vs 3.8 mm, $P=0.004$, adjusted for body surface area & age). This ethnic difference was not explained by diabetes or other measured risk factors. In men in the upper tertiles of CACS (23–2416 HU) there were no ethnic differences in LAD diameter (South Asian men: 3.0 mm, European men: 3.1 mm, $P=0.64$). Calcification scores were negatively correlated with LAD diameter in European men ($\rho=-0.38$, $P=0.016$) and not in South Asian men ($\rho=-0.06$, $P=0.72$).

Conclusions: The increased degree of LAD stenosis, despite similar symptoms and CACS scores, in South Asian men is attributable to narrower arteries. Advanced disease in Europeans is associated with inward remodelling, but not in South Asians.

PD.3. Angiotensinogen gene variants do not affect ambulatory blood pressure in the general population

P Braund, M Tobin, G Lavery, C Bodycote, S Raleigh, FJ Charchar, P Burton and NJ Samani

University of Leicester, Leicester, UK

Background: Conflicting association data between the angiotensinogen (AGT) gene and risk of hypertension could be caused by the level of salt intake. We analysed the effect of 8 AGT gene polymorphisms on 24 h BP (assessed as a quantitative trait) measured by an ambulatory approach in 1533 subjects in whom 24 h sodium intake was also assessed.

Methods and Results: The subjects comprised parents and adult offspring from 386 families recruited from the general population as part of the GRAPHIC (Genetic Regulation of Arterial Pressure of Humans in the Community) study. Ambulatory BP was measured using a Spacelabs 90207 monitor. Sodium intake was estimated from 24 h urinary sodium excretion. The AGT single nucleotide polymorphisms (SNPs) genotyped included 4 in the promoter region, 1 in intron 1, 1 in exon 2, 1 in intron 3 and 1 in exon 5. The SNPs were chosen to provide a full coverage of the gene at a D' of >0.8

and to include all SNPs with functional effects or associated with hypertension. All analyses took account of familial relationships and were adjusted for age, age² and sex as covariates. The minor allele frequencies for the SNPs ranged from 8.7% (rs11122575) to 40.2% (rs 5051). None of the SNPs showed a significant association with either ambulatory SBP or DBP analysed either for the full 24 h or for day-time and night-time periods. For 24 h SBP the 95% confidence intervals for the per allele BP effect excluded by the study ranged from -0.67 to $+0.91$ mm Hg for 5051 to -1.61 to 1.19 mm Hg for rs11122575. No significant interactions between salt intake and AGT SNPs were observed.

Conclusion: AGT gene variants do not significantly influence ambulatory BP phenotypes in the general population. Our findings do not support the concept that variants in the AGT gene are a risk factor for hypertension.

PD.4. The genetics of small vessel stroke: a meta-analysis of five candidate genes in over 16 000 subjects

R Rao, V Tah and P Sharma

Imperial College & Hammersmith Hospitals, London, UK

Background: Ischaemic stroke has a genetic basis. However, whether different subtypes of stroke have different genetic liabilities remains controversial with investigators arguing for an enhanced genetic contribution to small vessel cerebral ischaemia compared to large vessel disease. Most investigators have used the case-control model but these studies have been underpowered to reliably answer this question. We undertook the largest meta-analysis ever conducted to address this specific question.

Methods: We searched for all case-control association studies where ischaemic stroke had been analysed as a dichotomous trait. All gene molecular variants investigated with 3 or more papers were included in our analysis.

Results: Our search identified 526 papers where 145 polymorphisms had been tested from 110 candidate genes. Of these, five genes (ACE/ID, MTHFR/C677 T, ApoE/ε4, eNOS/4ab, PAI-1/4G5G) met our inclusion criteria, with 38 manuscripts in 16,073 subjects. Of the 5 genes investigated ACE/ID* was highly

significantly ($P < 0.00001$) and eNOS* significantly ($P = 0.03$) associated with small vessel but not large vessel disease.

Gene	Small vs Control (OR 95% CI)	Large vs Control (OR 95% CI)
ACE	1.56 (1.28–1.90)*	0.97 (0.79–1.19)
MTHFR	1.13 (0.94–1.37)	1.18 (1.00–1.39)
ApoE	0.68 (0.25–1.87)	0.91 (0.4–2.03)
eNOS	1.46 (1.05–2.03)*	1.07 (0.77–1.49)
PAI-1	0.90 (0.69–1.17)	0.84 (0.63–1.11)

Conclusions: Our results demonstrate that different subtypes of stroke may have different genetic aetiologies. This work emphasises the need to accurately clinical phenotype stroke patients in all future genetic studies. Our work has important implications for the genetics of cerebrovascular disease.

PD.5. Acute hemodynamic predictors of early outcome following acute stroke in treated hypertensive patients

N Shah¹, N Taub², T Black³, P Johnson⁴, R Panerai⁵, J Potter¹ and T Robinson¹

¹University of Leicester, Department of Cardiovascular Sciences, Ageing and Stroke Medicine Group, Leicester, UK; ²Trent Research and Support Unit, Department of Health Sciences, Leicester, UK; ³Royal Bournemouth Hospital, Bournemouth, UK; ⁴Department for Medicine, Royal Devon and Exeter Hospital, Exeter, UK and ⁵University of Leicester, Department of Medical Physics, Leicester Royal Infirmary, Leicester, UK

Background and purpose: Haemodynamic parameters including beat-to-beat blood pressure (BP), heart rate variability (HRV) and Cardiac Baroreceptor Sensitivity (BRS) are good long-term prognostic indicators following acute stroke. However, the predictive value of such parameters for early (2 weeks) outcome in previously treated hypertensive stroke patients is unknown as are the prognostic effects of previous antihypertensive treatment.

Methods: Ninety-one (50 male, mean age 72.2 ± 12.6) pre-existing treated hypertensive stroke patients from the COSSACS study were recruited. ECG, beat-to-beat BP recordings, cardiac BRS and HRV were recorded within 48 h of ictus. Early outcome was assessed as death and dependency (defined as modified Rankin score [mRS] of ≥ 2) on day 14 post-ictus.

Results: Mean baseline values for beat-to-beat SBP (1.6 mm Hg, CI -7.8 to 11.1 , $P = 0.74$), DBP (1.2 mm

Hg, CI -5.4 to 7.8 , $P = 0.72$), HRV (15.4 ms, CI -7.0 to 37.7 , $P = 0.17$) and cardiac BRS (4.2 ms/mm of Hg, 95% CI -1.2 to 9.5 , $P = 0.12$) at 2 weeks were similar between the independent and the dependent/dead groups. Regression modelling adjusting for baseline variables showed decreased HRV independently predicted an adverse outcome at 2 weeks ($P = 0.015$) as did higher beat-to-beat SBP ($P = 0.017$) and DBP ($P = 0.005$). Cardiac BRS and previous use of 'old' and 'new' antihypertensive agents were not however predictive of outcome.

Conclusions: The non-invasive assessment of haemodynamic parameters including beat-to-beat BP and HRV within 48 h of acute stroke onset have prognostic significance in predicting 2-week death/dependency, independent of stroke severity, disability and standard BP parameters. The effect of previous anti-hypertensive therapy in this small study did not appear to predict short-term outcome.