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LONG TERM EFFECT OF RENAL DENERVATION ON BLOOD PRESSURE IN PATIENTS WITH RESISTANT HYPERTENSION

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Renal sympathetic hyperactivity is seminal in the progression of hypertension (HTN). Catheter-based renal sympathetic denervation (RDN) has been shown to significantly reduce blood pressure in patients with hypertension (HTN). A critical issue is the long term durability of effect using this novel technique which has not yet been reported. A cohort of 45 patients with resistant HTN (SBP ≥160 mmHg on ≥3 anti-HTN drugs, including a diuretic) was originally reported. Here we report longer term data on these patients and similar patients subsequently treated with the catheter-based renal denervation in non-randomized studies. Office blood pressure (BP) data and safety data were available at 1, 3, 6, 12, 18 and 24 months post-procedure. A total of 153 pts were treated with catheter-based RDN at 19 centers in Australia, Europe, and the United States. Mean age was 57 ± 11 yrs; 39% were female; 31% diabetic, 30% with CAD. Baseline in values include mean office BP 176/83 ± 7/17 mmHg, mean of 5.0 anti-HTN medications, and eGFR 83 ± 20 mL/min/1.73m². The median time from first to last RF energy delivery was 38 minutes. The procedure was without complication in 97% (149/153) of cases. The major procedural complications included kidney euvuromys and one renal artery dissection, all managed without further sequelae. Post-procedure office BPs were reduced by 20/10, 24/11, 25/11, 23/11, 26/14, and 32/14 mmHg at 1, 3, 6, 12, 18 and 24 months respectively. One patient required stenting of a proximal renal artery stenosis that was present at baseline but grew by 6 months; no radiofrequency energy had been delivered in this location. Otherwise, there were no late adverse events associated with the therapy. We conclude that in patients with resistant hypertension, catheter-based RDN results in a substantial reduction in BP sustained out at least 2 years of follow-up, without significant adverse events.

GENOME-WIDE DISCOVERY OF GENES AND MIRNAs EXHIBITING DIFFERENTIAL EXPRESSION IN KIDNEYS OF ESSENTIAL HYPERTENSIVE PATIENTS AND CONTROLS

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Essential hypertension (EH) may involve, at least in part, alterations in the expression of particular protein-coding genes in the kidney. Besides protein-coding genes, the genome also contains miRNA genes (miRNA) that regulate the expression of these genes. Very little is, however, known about this mechanism in EH. Here we used a genome-wide approach to characterize expression differences in kidneys from EH compared with normotensive subjects obtained from the Silesian Renal Tissue Bank. RNA was extracted from medulla (n = 12) and applied to Affymetrix GeneChip® Human Gene 1.0 ST Arrays. After adjustment for age, we identified 13 genes whose expression differed between EH and normotensive kidneys (after correction by false discovery rate <0.16). In samples from older (>55 years) EH subjects, NR4A3 was the most highly over-expressed gene in EH (adjusted P = 0.006). Using quantitative PCR we validated expression changes for BTG2, DUSP6, HSPA1B, NR4A2, NR4A3 and SIK1. The protein encoded by SIK1 increases active cell sodium transport in response to elevated intracellular sodium. Interestingly, the expression of DUSP6, BTG2 and the NR4A transcription factor family is regulated by angiotensin II. Overexpression of DUSP6 predisposes to heart failure, and NR4A2 may play a crucial role in the transcriptional regulation of the aldosterone synthase (CYP11B2). An elevated aldosterone in some EH patients could be involved in EH. When coupled with functional studies as appropriate for each gene, our novel findings from cutting-edge array technology and bioinformatics should help in elucidation of the causes of EH and may provide new targets for treatment.

DEFINING THE PHENOTYPIC PATTERNS IN ESSENTIAL HYPERTENSION IS THE KEY TO IDENTIFYING IMPORTANT HIGH BLOOD PRESSURE GENES.

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The genes that cause or increase susceptibility to essential hypertension (EH) and related animal models are still unknown. Their identification is unlikely to be realised with current genetic approaches, because of enhanced ambiguities in the genotype-phenotype relationship in these polygenic disorders. The phenotype is not just an aggregate of traits, but must be related to specific components of the circulatory control system at different stages of EH. Hence, clues about important genes must come through the phenotype, reversing the order of current approaches. A recent systems analysis has highlighted major differences in circulatory control in the two main syndromes of EH: 1) stress- and salt-related EH (SSR-EH); 2) hypertensive obesity, i.e. SSR-EH + obesity. Each is initiated through sensitization of central synapses linking the cerebral cortex to the hypothalamic defence area. The result is a sustained increase in sympathetic neural activity at stimulating levels of this gene in 20 normal subjects. Subsequent progression of EH is largely through non-neuronal mechanisms, including changes in concentration of vascular autocoids (e.g. nitric oxide) and structural changes in large resistance vessels, which amplify rises in vascular resistance. These result in increased blood flow heterogeneity with rarefaction and deterioration of vital organs. SSR-EH also increases food intake in response to stress, but only 40% of these individuals develop hypertensive obesity. Their brain ignores the adiposity signals that normally reduce eating. The hyperinsulinemia masks the sympathetic vasoconstriction through its diactor action and raises blood volume through its renal action, whilst diabetic complications are common. In each syndrome the neural and non-neuronal determinants of hypertension provide targets for identifying high BP genes. To identify the important genes will require new approaches, probably similar to those used in developmental genetics; transgenic technology may help validate hypotheses and determine whether an observed effect involves single or multiple physiological mechanisms. To obtain answers will require substantial collaborative efforts between physiologists and geneticists. The beneficiary will be integrative biology.

DIETARY INTERVENTION FOR THE DEVELOPMENT OF EXPERIMENTAL MODELS OF DIABETIC CARDIOMYOPATHY

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Diabetes increasing in prevalence and is associated with an increased risk of cardiovascular disease, in particular diabetic cardiomyopathy (DCM). There are a number of genetic models for the development of DCM however these fail to take into account the environmental influences which are central to its progression. This study thus aims to investigate and characterise cardiac function in diet-induced animal models relevant to type 2 diabetes (T2D). Sprague-Dawley rats received either control standard chow (0% fat; n=19), or one of three experimental diets, high fat diet (HFD, 45% fat; n=17), fructose diet (10% fructose water; n=17) or HFD plus low dose streptozotocin (HFD/STZ 15mg/kg; n=16) for 20 weeks. Body weight (BW), food intake, fasted blood samples, insulin (ITT) and oral glucose tolerance testing (OGTT) were recorded periodically. Cardiac function was assessed via echocardiography and pressure volume (PV) conductance catheter at 20 weeks. Results are versus control. Fructose fed rats had a significantly higher energy intake (P = 0.002) but no change in BW. Cardiac contractility in fructose fed rats was impaired as shown by decreased fractional area change (P = 0.026) and fractional shortening (P = 0.036) from echocardiography. HFD fed rats had a 9% greater body mass with increased retroperitoneal (P < 0.001) and epididymal adiposity (P < 0.001) due to higher caloric intake (P = 0.039). HFD rats were insulin resistant (P = 0.011), and HFD/STZ rats were glucose intolerant (P = 0.001) at 20 weeks. Final fasting blood glucose (FBG) of HFD/STZ rats (P < 0.001) was higher than HFD alone. A significant improved relaxation with decreased E/A ratio (P = 0.010). Cardiac function assessed by gold standard PV conductance under baseline and dobutamine-stress conditions however showed no differences between the groups. In conclusion, insulin resistance and glucose intolerance in these diet-induced models of T2D was not translated to impaired diastolic or systolic dysfunction and their future use should be approached with caution.

ENDOTHELIAL OVEREXPRESSION OF ARGINASE II INDUCES ENDOTHELIAL DYSFUNCTION AND PROMOTES ATHEROSCLEROSIS

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Arginase, found in the vasculature as arginase I and II competes with endothelial nitric oxide synthase (eNOS) for L-arginine and can regulate nitric oxide (NO) production. Arginase I and II are both implicated in the endothelial dysfunction of many disease states. We have previously shown that arginase I overexpression may have cardioprotective effects; we now aim to elucidate the contribution of arginase II to endothelial function and atherosclerosis. Mice on a C57BL/6J background with endothelial specific overexpression (under the control of the Tie2 promoter) of the human arginase II gene and their wild type (WT) littermate controls were utilized. Arginase activity was elevated in all organs in the transgenic arginase II (Tie2-ArgII) mice except the liver. Endothelial specificity was confirmed with no change in arginase activity in peritoneal macrophages. Plasma levels of lipids and L-arginine and its metabolites and
vacular reactive oxygen species levels were unaltered. Using small vessel myography, aortic responses to acetylcholine were diminished in the Tie2ArgII mice (pEC50 = -7.1 ± 0.1, \( R_m = 81.6 \pm 5\), n = 7) when compared to their WT littermate controls (pEC50 = -7.6 ± 0.1, \( R_m = 90.5 \pm 5\), n = 5. \( P < 0.05 \)) suggesting endothelial dysfunction. Upon analysis of the aorta of the Tie2ArgII mice by PCR, mouse arginase I and II expression were unaltered, suggesting no compensatory response to the overexpression of human arginase II gene. However, gene expression of eNOS (WT: Fold 8; P < 0.001) and inducible NOS (NOS; WT: Fold 1.3 ± 0.4, n = 6; vs Tie2ArgII; Fold 6.7 ± 1.4, n = 8) was elevated. When these mice were crossed with ApoE deficient mice and placed on a high fat diet, there were also increased lesions in the aorta of the Tie2ArgII mice compared to the ApoE deficient controls (Tie2ArgII: 26% vs ApoE deficient: 21%; n = 11; \( P < 0.05 \)). Taken together, these findings suggest that endothelial overexpression of arginase II in mice is detrimental to vascular function and promotes atherosclerosis possibly by regulating the expression of function of NOS.

6 SELECTIVE SEROTONIN REUPTAKE INHIBITOR ANTIDEPRESSANTS MAY INTERFERE WITH THE DIAGNOSTIC WORK UP OF PRIMARY ALDOSTERONISM

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Background: Plasma aldosterone/renin ratio (ARR) is the most popular screening test for primary aldosteronism (PAL). Antidepressants affect functions which control the secretion of aldosterone and renin and therefore may alter ARR. We are unaware of previously published data on the effects of antidepressants on ARR.

Methods: Normotensive, depressed inpatients (n = 26) underwent measurement (measured, ambulatory) of plasma aldosterone, direct renin concentration (DRC), renin activity (PRA), electrolytes and creatinine and urinary aldosterone, cortisol, electrolytes and creatinine at baseline, and after two weeks and six weeks treatment with sertraline (n = 14) or escitalopram (n = 12).

Results: For both antidepressants, treatment was associated with rises in aldosterone (sertraline: baseline mean = 243 ± 34, 2 weeks 256 ± 33, 6 weeks 267 ± 34 pg/mL (P = 0.01 by ANOVA); escitalopram: 261 ± 38, 269 ± 38, 282 ± 40 pg/mL (P < 0.05)), DRC [19.5 ± 2.2, 33.3 ± 2.5, 39.0 ± 2.4 μU/L (P < 0.001)], PRA [24.5 ± 3.4, 34.0 ± 2.7, 42.4 ± 2.8 μU/mL (P < 0.001)] and PRA [24.2 ± 0.21, 25.8 ± 0.26, 46.8 ± 0.42 ng/mL/h (P < 0.001)], 4.31 ± 0.22, 5.57 ± 0.36, 6.42 ± 0.53 ng/mL/h (P < 0.001)]. ARR fell significantly whether calculated using DRC [sertraline 13.7 ± 2.2, 7.5 ± 0.7, 6.8 ± 0.7 (P < 0.001)]; escitalopram 11.5 ± 1.9, 8.0 ± 1.1, 6.6 ± 1.0 (P < 0.001)]; or PRA [116.6 ± 15.8, 108.4 ± 15.6, 60.4 ± 6.2 (P < 0.001)]; 61.2 ± 6.1, 50.0 ± 7.7, 45.8 ± 8.0 (P < 0.001)].

Conclusion: Selective serotonin reuptake inhibitor antidepressants can significantly reduce ARR and therefore potentially increase the risk of false negative results when screening for PAL. Further studies in hypertensive patients are required.

7 KNOCKDOWN OF ANGIOTENSINENGEN PROTEIN IN VIVO

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The renin-angiotensin system (RAS) is defined by its ability to potently regulate blood pressure and fluid homeostasis. This system is made more complex by the existence of several potentially independent tissue RAS, notably in the brain and heart. Current methods are not adequate to resolve the relative importance and anatomy of the tissue RAS in vivo without confounding influence by systemic RAS. A novel approach is to knockdown endogenous gene expression of RAS components in specific cells of a single brain region. To do this, we exploited current RNA interference technology whereby short-hairpin RNA, complementary to a region of the gene of interest, binds to the cell mRNA causing it to be degraded by endogenous DICER enzyme. We designed a microRNA (mir) sequence to complement a region of the rat angiotensinogen (Ao) gene. We have previously shown in vitro that this mir reduces Ao protein by more than 90%. The mir primer was then ligated into a lentiviral expression plasmid vector and expression directed towards astrocytes, the main Ao synthesizing brain cells, using an astrocyte-specific GFAP(B)3 promoter and a mokola coat protein. As a negative control, we inserted in the carotid and femoral arteries and positioned in the thoracic aorta at a known insertion site. The coefficients of the polynomial are shown in Table PWV of LPK was consistently higher with an average of 1.5m/sec at all pressures. The quantification of PWV-MAP phase plots showed an increase in isobaric arterial stiffness, suggesting pathology in the arterial system preceding the development of end-stage-renal failure in LPK. This may be a contributing factor to the high incidence of cardiovascular disease in PDK and determination of arterial stiffness may be an important prognostic indicator and treatment goal, in addition to decreasing vascular resistance. [0] Phillips JK et al, Blood Press Res. 2007;30(3):129-44.

8 FAILURE OF THE SUPEROXIDE DISMUTASE MIMETIC TEMPO TO REDUCE ARTERIAL PRESSURE AND DELAY DISEASE PROGRESSION IN A RAT MODEL OF POLYCYSTIC KIDNEY DISEASE

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There are currently no effective therapeutic strategies for patients with polycystic kidney disease (PKD). Superoxide dismutase mimetics such as tempol have shown promise for the treatment of hypertension and kidney disease associated with oxidative stress. PKD is also associated with renal oxidative stress. In the current study we examined the effects of chronic tempol treatment on arterial pressure and disease progression in a rat model of autosomal recessive PKD. Tempol was administered in drinking water (1 mM) from 4-13 weeks of age (n = 5 per group). At 13 weeks of age PKD rats had grossly enlarged kidneys (WK = left kidney), elevated mean arterial pressure (MAP) and much greater urine flow (UVOL) and plasma urea (urea), than control animals (Tables Histological and immunohistochemical analysis of renal tissue revealed the presence of extensive cyst formation throughout the renal cortex and medulla of PKD rats, derived from collecting ducts and both distal and proximal tubules. The cysts were lined by a flattened epithelial layer with evidence of tubulointerstitial matrix expansion and the accumulation of interstitial collagen. Chronic tempol treatment had no appreciable effects on these indices of disease progression. Our findings provide no support for the proposition that superoxide dismutase could provide an effective therapeutic approach in familial PKD.

9 ISOBARIC AORTIC PULSE WAVE VELOCITY IS INCREASED IN A NOVEL RODENT MODEL OF AUTOSOMAL RECESSIVE POLYCYSTIC KIDNEY DISEASE

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The Australian School of Advanced Medicine, Macquarie University, Sydney, Australia. The Lewis polycystic kidney disease rat (LPK) is a novel animal model of end-stage renal failure (ESRF) [1]. The hypertensive status of these animals is well established; however the functional changes in arterial stiffness, determined by pulse wave velocity (PWV) are unknown. The present study investigated vascular and fluid changes in LPK through quantification of the PWV – mean arterial pressure (MAP) relationship. Experiments were performed on urethane anaesthetised (1.3 g/kg) LPK (n = 6) and control Lewis (n = 6) rats at 12-13 weeks of age by recording beat-to-beat PWV and MAP using two high fidelity 1.4F catheter-tipped pressure sensors inserted in the carotid and femoral arteries and positioned in the thoracic aorta at a known distance apart. Pressure was increased and decreased by infusion of phenylephrine and sodium nitroprusside respectively. PWV – MAP phase plots were obtained for pressure in the range of 60 – 210 mmHg. The PWV-MAP relationships showed a similar pattern in both LPK and controls and were expressed in a quadratic polynomial such that PWV = aMAP2 + bMAP + c. The coefficients of the polynomial are shown in Table PWV of LPK was consistently higher

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>PKD</th>
<th>PKD + Tempol</th>
</tr>
</thead>
<tbody>
<tr>
<td>WK (g)</td>
<td>1.46 ± 0.07</td>
<td>12.6 ± 0.7</td>
<td>13.1 ± 0.2</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>103 ± 1</td>
<td>159 ± 11</td>
<td>146 ± 15</td>
</tr>
<tr>
<td>UVOL (ml/24 h)</td>
<td>15.8 ± 0.5</td>
<td>45.5 ± 3.3</td>
<td>48.5 ± 3.2</td>
</tr>
<tr>
<td>[urea] (mM)</td>
<td>5.3 ± 0.8</td>
<td>27.4 ± 7.2</td>
<td>27.7 ± 5.5</td>
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VULNERABILITY OF INTRAUTERINE GROWTH RESTRICTED RAT OFFSPRING TO ADULT HYPERGLYCEMIA: EFFECTS ON RENAL FUNCTION

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Intrauterine growth restriction (IUGR) leads to a reduction in nephron endowment at birth and is strongly linked to renal dysfunction in adulthood. We therefore hypothesised that IUGR offspring would be more vulnerable to secondary postnatal insults. The aim of this study was to investigate whether a postnatal insult of hyperglycemia in rats leads to greater renal dysfunction in IUGR rat offspring. Female WKY rats were fed either a normal protein diet (NPD, 20% casein) or low protein diet (LPD, 8.7% casein) during pregnancy and lactation. At 23 weeks of age, all rats were stereotaxically injected with saline or 3.5mg/kg insulin (prophylaxis) and blood glucose levels in diabetic rats were maintained at below 10mmol/L, the deleterious effects on renal function were markedly attenuated and GFR/g of kidney tissue was restored (P < 0.001).

Throughout the experimental period (P < 0.001). Offspring with hyperglycemia exhibited increased kidney weight relative to BW in both the mild and moderate groups (P < 0.001). There were no significant differences in blood pressure, heart rate and GFR (adjusted for kidney weight) between LPD offspring and NPD offspring at 32 weeks of age. However, there was a significant decrease in GFR in all hyperglycaemic offspring (P < 0.001). RVR was increased (P = 0.005) in LPD offspring and this was exacerbated in hyperglycemic offspring (P < 0.0001). However, when the blood glucose levels in diabetic rats were maintained at below 10mmol/L, the deleterious effects on renal function were markedly attenuated and GFR of kidney tissue was restored to normal even in the presence of a congenital nephron deficit. In conclusion, IUGR leads to altered renal function in adulthood and heightened vulnerability to hyperglycemia, however, tight glycemic control attenuates the adverse effects of hyperglycemia in all groups.

REDUCED URINE CONCENTRATING ABILITY IN RESPONSE TO VASOPRESSIN AND WATER DEPRIVATION IN YOUNG AND AGING UNINEPHRECTOMISED MALE SHEEP

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Urine concentrating ability declines with age predisposing to renal failure, likely associated with impairments in arginine vasopressin (AVP) secretion or reduced sensitivity of collecting ducts (CDD) to AVP due to reductions in aquaporin-2 (AQP-2). We have reported that fetal uninephrectomy (uni-x) at 100d gestation (term≈150d) results in decreased arterial mean pressure (MAP) and lower glomerular filtration rate (GFR) in 6 mth male sheep. This reduced renal function maybe associated with impaired urine concentrating ability of the remnant kidney to 1) non-pressure dose of exogenous AVP (0.2 μg/kg i.v.) and 2) modest 30 h period of water deprivation and that these responses may worsen with age. Cardiovascular (MAP) and renal functional (GFR and urine flow rate) parameters were measured prior to and during AVP infusion or water deprivation in conscious male sheep at 6 mth and 4 y. Basal MAP increased with age in both groups (Pgroup×age<0.001) in both groups compared to the sham group (6mth: 8±3 mmHg, 4 y: 12±2 mmHg, Pgroup<0.05, Page<0.046). Basal GFR was lower in uni-x compared to sham group at both ages (6 mth: 26±2%; 4 y: 33±2%; P<0.001). GFR declined with age in both groups with uni-x having greater decrease with age compared to sham (16±1%; uni-x: 26±2%; P<0.001, Pgroup×age<0.001). UFR decreased with age in both groups (Pgroup<0.001) but was similar between groups at both ages. Uosm increased similarly in both groups with age (P<0.005). Uni-x animals had lower maximal Uosm in response to AVP at both ages compared to sham (6 mth: 3210±96, 4 y: 2777±34, uni-x: 6 mth: 2522±23, 4 y: 1712±35, P<0.001, Pgroup<0.001) and the decrease in maximal Uosm in response to AVP was greater in uni-x than sham with age (sham: 13±3%, uni-x: 32±2%, P<0.001). Responses to dehydration were similar to that of AVP infusion in both groups at both ages. Uni-x animals had reduced renal expression of AQP-2 (P<0.01). A low nephron endowment increases the risk of hypertension and chronic renal disease and may incur greater vulnerability to physiological challenges such as water deprivation as observed in the uni-x animals.

TIME COURSE OF ACTIVATED MICROGLIA FOLLOWING MYOCARDIAL INFARCTION IN RATS

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Cardiac microglia are activated during the establishment phase of heart failure. In the hypothalamic paraventricular nucleus (PVN), microglial activation in the PVN, but this does not occur immediately post MI, and microglia are activated during the establishment phase of heart failure.

CARDIOPROTECTIVE ACTION OF MINERALOCORTICOID RECEPTOR ANTAGONISTS DURING EXPERIMENTAL MYOCARDIAL INFARCTION: ROLE OF APOTOPSIS REPERSONSER WITH CASPASE RECRUITMENT DOMAIN (ARC)

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Clinical studies show mineralocorticoid receptor (MR) antagonists reduce morbidity and mortality in heart failure and myocardial infarction. Plasma levels of aldosterone were in the low/normal range and doses of MR antagonists were low, raising questions over the mechanism for the cardioprotective action of MR antagonists. Apoptosis plays an important role in post-infarction left ventricular remodelling, therefore we examined whether MR antagonists modulate cardiomyocyte death via suppressing apoptosis. Hearts from adult male Sprague Dawley rats were subjected to regional ischemia followed by reperfusion ex vivo. MR antagonists were added to perfuse prior to ischemia. After reperfusion, left ventricular tissues were frozen for molecular analysis of apoptotic mediators. Perfusion with spironolactone (10 nm) significantly reduced infarct size (35±2%, n = 7 vs 44±1%, n = 6; P < 0.05). Apoptosis is regulated by the balance between pro- and anti-apoptotic proteins during reperfusion injury. Spironolactone prevented ischemia-reperfusion (IR)-induced activation of caspases-3, -8 and -9 (see Fig 1), whereas there was no change in caspase-8 processing.

PRETERM BIRTH HAS INJURIOUS AND PERSISTENT EFFECTS ON THE STRUCTURE AND COMPOSITION OF THE AORTA AND PULMONARY ARTERY

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Background: Preterm birth affects 9-12% of live births and the incidence is increasing. Epidemiological studies have linked preterm birth to the development of elevated blood pressure and aortic narrowing in later life, suggesting altered arterial development. Our

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**Blood pressure, measurement of post-prandial glucose. Data expressed as mean ± SEM.**

**Subjects with Isolated Clinic Hypertension**

GLUCOSE INTOLERANCE, A CARDIOVASCULAR RISK BIOMARKER IN SUBJECTS WITH ISOLATED CLINIC HYPTERTENSION

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1 Department of Vascular Sciences, Southern Health; 2 Monash Heart, Southern Health; 3 La Trobe University; 4 Monash University

Patients with isolated clinic hypertension (ICH) are more likely to develop sustained hypertension (HT) and blood glucose abnormalities than their normotensive (NT) counterparts. In this study 36 NT, 33 ICH and 40 untreated HT subjects underwent an oral glucose tolerance test, aorto-femoral pulse wave velocity (PWVaf) and blood tests, to assess if a biomarker(s) associated with increased cardiovascular risk could be identified in ICH. Subjects underwent 24-hour ambulatory blood pressure (ABPM) monitoring. Insulin resistance was assessed by four measures (glucose area-under-the-curve (glucose AUC), 2-hour glucose post dextrose load (2hPG), HOMA (fasting insulin × fasting glucose)/22.5) and insulin area-under-the-curve (insulin AUC).

**Conclusion:** Moderately preterm birth has injurious and persistent effects on the structure and composition of the aorta, with lesser effects in the pulmonary artery. Our findings suggest that individuals born preterm may be at increased risk of atherosclerosis and aortic aneurysms.

**Preterm Birth and Aortic Aneurysms**

Using an established ovine model of preterm birth, lambs were born at 114% of gestation and underwent necropsy at 11 weeks after birth; controls were born at term. In preterm lambs we found injury in the aorta but not in the pulmonary artery; controls were unaffected. Preterm lambs had significantly thicker aortic walls and a smaller mean area. Analysis of aorta only.

**Conclusion:**

- Preterm birth has injurious and persistent effects on the structure and composition of the aorta, with lesser effects in the pulmonary artery.
- Our findings suggest that individuals born preterm may be at increased risk of atherosclerosis and aortic aneurysms.

**Evaluation of Total Cardiovascular Risk in Subjects with ICHT**

- There were no significant differences between groups for markers of inflammation or endothelial dysfunction.
- Assessment of heart rate response to deep breathing, valsalva and standing should be age adjusted, whereas blood pressure response to the grip test should be gender adjusted.

<table>
<thead>
<tr>
<th>NT</th>
<th>ICHT</th>
<th>HT</th>
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<tbody>
<tr>
<td>Age years</td>
<td>54.9 ± 9.3</td>
<td>56.2 ± 8.7</td>
</tr>
<tr>
<td>Female n (%)</td>
<td>26 (72.2)</td>
<td>27 (81.1)</td>
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<tr>
<td>BP mmHg</td>
<td>121/80 ± 9/8</td>
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<td>day AMBP mmHg</td>
<td>127/72 ± 6/7</td>
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<td>2hPG mmol/L</td>
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<td>6.9 (6.2-7.7)</td>
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<td>PWVaf m/s</td>
<td>7.8 (7.4-8.2)</td>
<td>8.1 (7.7-8.5)</td>
</tr>
</tbody>
</table>

**Bedside Autonomic Function Normal Values Should Be Age or Gender Adjusted**

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In 1985 Ewing published normal autonomic function data using a series of bedside tests. In this study we further refine normal autonomic function data by testing for and excluding subjects at greater risk of autonomic dysfunction. We also examine autonomic function across blood pressure categories. Subjects were initially excluded if they were on antihypertensive treatment, reported heavy alcohol use, had known diabetes or on any medication that may interfere with autonomic function. Subjects underwent 24 hour ambulatory blood pressure monitoring and an oral glucose tolerance test. Subjects found to have impaired fasting glucose, impaired glucose tolerance or diabetes were then excluded. Skewed data was transformed for analysis. Deep breathing, valsalva ratio and 30:15 ratio were all significantly related to age (P < 0.001). Only the blood pressure response to grip (P = 0.09) appeared to have a relationship to gender although not statistically significant. There was no significant difference in heart rate response to deep breathing, valsalva and standing should be age adjusted, whereas blood pressure response to the grip test should be gender adjusted.

**Effects of the Endpoint Adjudication Process on the Results of the Clinical Trial: The ADVANCE Study**

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Endpoint adjudication committees (EPCs) are widely used in clinical trials. However, it remains uncertain whether the endpoint adjudication process really improves the precision and reliability of the treatment effects reported. The aim of the present analysis is to assess the effects of the endpoint adjudication process on the main findings of the Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified Release Controlled Evaluation (ADVANCE) study. The ADVANCE study was a multicentre, factorial randomised controlled trial which investigated the effects of blood pressure lowering and intensive blood glucose control in 11,140 patients with type 2 diabetes. Participants were randomly assigned, in a factorial design, to perindopril-indapamide or matching placebo and to either an intensive or a standard glucose control strategy. Primary outcomes were composites of macrovascular (nonfatal myocardial infarction, nonfatal stroke, or cardiovascular death) and microvascular (new or worsening nephropathy or retinopathy) events. Suspected primary outcomes were initially reported by the site investigators at the 215 centres with subsequent adjudication by the EPC. Overall, over a median follow-up of 5 years, the site investigators reported one or more primary macrovascular or microvascular outcomes for 2,443 participants. After confirmation of 2,077 (85%) events and inclusion of additional 48 events, 2,125 events were finally diagnosed by the EPC. The estimated relative risk reductions (95% confidence intervals) of the primary macrovascular or microvascular outcomes for the blood pressure lowering comparison were 8% (1-15%) based on the investigator-reported events and 9% (0 to 17%) based on the EPAC-based events (P for homogeneity = 0.70). The corresponding findings for the glucose lowering comparison were 8% (1-15%) and 10% (2% to 18%) (P for homogeneity = 0.60). The relative risk reductions were also highly comparable when studied separately for macrovascular and microvascular events for both comparisons (all P for homogeneity > 0.6). In conclusion, the endpoint adjudication process had little impact on the main findings for microvascular events as well as macrovascular events in the ADVANCE study.

**Effect of Diabetes and Macular Degeneration on Optimality Parameters of Retinal Vascularization**

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Arterial diameters and angles at bifurcation points follow optimality principles to minimize energy losses, shear stress and intra-vascular volume in healthy vascular networks. The retinal microvascularization is readily visualized non-invasively and quantified by image processing of fundus images. Previous studies have shown that retinal arteriolar bifurcation angles are narrower in hypertension, older age and low birth weight. This study examined diabetes and age related macular degeneration (AMD). These diseases are major causes of blindness and may be associated with localised retinal vascular changes. Fundus images from patients with diabetes (n = 5) and AMD (n = 5) were captured using a Canon retinal camera. Thirty bifurcations of diabetic eyes and 30 of AMD were marked manually along the major superior and inferior arterial vascular arcades. The diabetics ranged from no retinopathy to proliferative, while the AMD cases were all cases with extensive soft drusen, one with a proliferative, while the AMD cases were all cases with extensive soft drusen, one with a...
significantly different from healthy controls (0.10 ± 0.18) reported in the literature (P < 0.01 for
diabetes and P = 0.011 for BMI cases). Blood sugars were not significantly different from
published normal values. Further analysis including additional subjects is required to correct for age,
severity of disease and to evaluate optimality with respect to distance from the optic disc.

RESPONSIVENESS OF ALDOSTERONE TO UPRIGHT POSTURE, EVEN WHEN
MEASURED BY A HIGHLY ACCURATE MASS SPECTROMETRIC METHOD,
CANNOT REPLACE ADRENAL VENOUS SAMPLING IN THE DIFFERENTIATION
OF UNILATERAL AND BILATERAL FORMS OF PRIMARY ALDOSTERONISM
(PAL)

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Background: Differentiating unilateral (U-) from bilateral (B-) PAL is important as they are
treated differently. While adrenal venous sampling (AVS) is the most reliable approach, less
invasive alternatives have included measuring the response of aldosterone (aldo) to upright
posture. We revisited this issue utilising a new, highly accurate mass spectrometric aldosterone
assay.
Methods: Plasma aldo was measured by HPLC tandem mass spectrometry (1) in two control
subjects with essential hypertension at 0800h after overnight recumbency and hourly until
14:00h following assumption (at 0800h) of upright posture to determine the time course of the
posture response and (2) in 83 patients with B-PAL, 30 with U-PAL and four control subjects
at 0700h after overnight recumbency and at 1000h following 3h of upright posture. Patients
taking interfering medications and those showing a rise in cortisol between 0700h (or 0800h) and
1000h were excluded.
Results: (1) In two control subjects, plasma aldo demonstrated maximal responses at 3 and
4 hours after assuming upright posture. (2) In 4 controls, aldo (3h) rose by 76, 100, 145 and
867% respectively. (3) Mean (± SD) % responsiveness for B-PAL was 138±122% (mean aldo
corrected to 71 ± 32 and upright 146 ± 57 pmol) and for U-PAL was 69±124% (160 ± 122 and
230 ± 202 pmol) (P<0.05) (4) Using a 50% rise above basal to define responsiveness, 90% of
patients with B-PAL and 53% with U-PAL were responsive (P<0.01). Selection of other criteria
(from 10-70%) to define responsiveness did not improve discriminative power (see table). In no subject with B-PAL [compared with 9 (50%) with U-PAL; P<0.0001] did upright aldo rise by at least 50% below basal.
Conclusion: Demonstration of a 50% rise in aldo over basal following 3h of upright posture
after overnight recumbency is more in keeping with B-PAL than U-PAL, but the degree of
overlap renders this test insufficiently discriminating to serve as a substitute for AVS. A fall in
aldo of at least 30% virtually excludes B-PAL.

EFFECT OF CONTRACEPTIVES ON ALDOSTERONE/RENIN RATIO MAY VARY
ACCORDING TO THE COMPONENTS OF CONTRACEPTIVE, RENIN ASSAY
METHOD AND ROUTE OF ADMINISTRATION

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Background: The most popular screening test for primary aldosteronism (PAL) is the plasma
aldosterone/renin ratio (ARR). Because both estrogen and progesterone affect aldosterone and
renin levels, we studied effects of two types of contraceptives on ARR, measuring renin as both
direct (DRC) and plasma renin activity (PRA) Methods: Normotensive, healthy women (n=32)
derived urine (mean and to aldosterone, cortisol, electrolytes and creatinine and urinary aldosterone,
corticosteroids, electrolytes and creatinine at baseline, and after (1) one and three weeks after
commencement of Yaz (n=17) [combined oral contraceptive pill (OCP), ethinylestradiol and
drospirenone], (2) one or six weeks after implantation of Implanon (n=15) [sub dermal
tetraestrogens, third generation progestogen]. Results: Treatment with Yaz was associated with rises in aldosterone [baseline median (range)
131 (85-590), 1 week 200 (130-784), 3 weeks 412 (199-1010) pmol/L, P = 0.001 by
Friedman Test] and PRA [21.1 (2.4-7.2), 3.6 (1.5-7.1), 4.9 (1.5-10.8), P = 0.001]; decreases in DRC
[22 (11-36), 21 (8.7.41), 14 (8.5-39) mL/L, P = 0.001] and increases in ARR calculated by
DRC [6.8 (3.3-31.3), 10.9 (5.2-58.9), 29.8 (6.1-8.5), P<0.001]. There were no changes in
PRA calculated by PRA, plasma electrolytes and creatinine, or all urinary measurements.
After Implanon insertion, there was no significant change in any measured parameter.
Conclusion: Yaz (Drospirenone/ethinyl estradiol) increases ARR and risk of false positive
results during screening for PAL if DRC is used to calculate the ratio, while Implanon has no
effect.

DIETARY SODIUM AND ALDOSTERONE LEVELS ARE RELATED TO SEVERITY
OF OBSTRUCTIVE SLEEP APNEA IN PATIENTS WITH RESISTANT
HYPERTENSION

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Background: Salt appetite is a motivated behavioural state that drives animals to seek and ingest foods
and fluids that contain sodium. Although experimental studies have demonstrated that aldosterone
stimulates salt ingestion, the role of aldosterone in determining salt appetite in humans is unknown.
To explore the role of aldosterone in contributing to salt appetite in humans by evaluating patients
with aldosterone-producing adenoma (APA) before and after adrenalectomy (ADX), Patients with APA (n=21) were admitted for fluorocortisone suppression testing (FST), before and 7.7±5.5 months after ADX. FST has a very strict protocol and is performed to definitively confirm or exclude primary aldosteronism. During FST, patients are admitted to ingest a high-salt diet (including Slow Na 30 mmol tds and free access to dietary salt) and fluorocortisone for 5 days. We compared urinary sodium (UNa) and volume (Uvol), collected on the last day (day 4) of FST, using exactly the same protocol, before and after ADX. Plasma aldosterone, aldosterone/renin ratio and urinary aldosterone significantly decreased and renin increased after ADX as expected. UNa and Uvol significantly decreased from 292.0±72.9 mmol/day to 241.5±46.4 mmol/day (P = 0.0063) and from 2949±981 ml/day to 2064±574 ml/day (P = 0.0003), respectively, despite equal salt supplementation. Aldosterone excess in humans may contribute to salt appetite and its correction by ADX seems to reduce salt intake.

INCREASED SYSTEMIC VASCULAR RESISTANCE IS ASSOCIATED WITH THE
DECLINE IN CARDIAC FUNCTION IN NORMAL PREGNANCY NEAR TERM

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We have shown a decline in cardiac function in normal pregnancy towards term. In this study we
analysed the hemodynamic characteristics measured at the time of our original study to identify factors that might explain the cardiac dysfunction. Subjects were nulliparous pregnant women (n = 32) seen in early (median 16 weeks) and again in late (median 37 weeks) pregnancy. Pulse wave analysis using the Sphygmocor device provided estimates of central mean arterial (cMAP) and pulse (cPP) pressures, augmentation index (AIc) and pulse wave velocity (PWV). Echocardiography was used to determine cardiac output (CO) and myocardial strain (MS) from 2D speckle tracking. Systemic vascular resistance was calculated as CO/cMAP. Data are median (interquartile range), with paired non-parametric (Wilcoxon) statistical comparison. At 37 weeks, a significant reduction in cardiac function, evident as a fall in MS, was paralleled by increases in central pressures and SVR.

In a multiple regression analysis with MS at 37 weeks as the dependent variable and age, CO, Alc at 37 weeks and MS at 16 weeks as explanatory variables. We found significant independent correlation between MS and SVR at 37 weeks (β = 0.006, beta = −1.17). As SVR influences cMAP directly and cPP through increased pulse wave reflection, it is potentially an underlying cause for increased pressure load and reduced cardiac function towards the end of normal pregnancy. The explanation for the change in SVR merits further study.
Hypertension involves alterations in expression of genes responsible for both the onset and also the established phases of raised blood pressure. Here we applied an integrated genome-transcriptome approach to identify genes whose expression is altered in early and late phases of hypertension in the spontaneously hypertensive and Lyon hypertensive rat strains. Global gene expression changes in kidney, adrenal, heart and artery, available on public databases, were subject to a combinatorial meta-analysis and data were used to identify common pathways. To identify genes responsible for the onset and maintenance of hypertension we used a new, improved statistical approach that adjusted for between-platform differences using the Bioconductor approach of Speed and Gagnon-Bartsch at UC-Berkeley, and a new statistical method developed by co-author Yang which eliminated expression differences that were related to ageing rather than hypertension. A total of 74 microarray experiments were included. Our statistical analysis identified 29 genes whose expression differed between the pre-hypertensive and hypertensive phases. Genes, such as Ccl2, Apoe, Eptn2, Eno1, Spool and Up1 stood out as relevant to the onset of hypertension. After Bonferroni correction (P<0.05) and a change of at least 20%, 140 gene expression differences were identified in the maintenance phase of hypertension. These included Adra1d, Apoe, Cc2, Cc16, Dusp5, Gata3, Lpl, Nppb, Npy, Sdc3, Eno1, Mtx1, Pnm, Pik3r3, Pla2g2a, Postn and Thbd4. The new method confirmed most genes which we identified in our meta-analysis published in Aug 2010 issue of Hypertension. Gene set enrichment analysis indicated over-representation of terms known to be important for hypertension, such as inflammatory response, regression of endothelial cell proliferation, response to stress, angiogenesis, response to oxidative stress and nitric oxide levels. Our meta-analysis has revealed the global gene expression altered in early and late hypertension. These data reveal potential causative and maintenance mechanisms and pathways.

**GENOME-WIDE ASSOCIATION STUDY OF RETINAL ARTERIOR DIMENSIONS AND INTERACTION WITH CORONARY RISK FACTORS**

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Structural narrowing in small arteries and arterioles of the retina is a marker of hypertension. We describe a genome-wide association analysis of retinal arterial diameter in subjects from the Blue Mountain Eye Study (BMES), a population-based study of older Australians. Retinal photographs were used to measure the calibre of the retinal arteries using a validated computer-assisted method and summarized as the “central retinal artery equivalent” (CRAE), representing the average retinal arteriolar diameter of the eye. Genotyping data were available in 2761 subjects (the Illumina 610K). After quality control filtering, imputation from the 1000genomes project and exclusion of subjects with positive or uncertain diabetes status, this analysis included 54402 SNPs in each of 2010 (1191 females, 841 males) subjects of median age 66 y (IQR 14) with mean CRAE of 168.2 (SD: 15.3). Association testing under an additive model was performed using PLINK, adjusted for age, sex and ancestry (Model 1). A total of 130 SNPs achieved a likelihood ratio P-value of < 10\(^{-6}\). Prominent putative loci were identified on 17 chromosomes. Further analyses were performed with additional adjustments for systolic blood pressure (SBP), body mass index (BMI) and smoking status (Model 2) included 1910 subjects. Model 2 resulted in 95 SNPs achieving a P-value of < 10\(^{-8}\) that included suggested loci on 16 chromosomes. Many of these loci found from Model 2 corresponded to those found from the Model 1. However, suggested loci on chromosomes 2 and 7 by Model 1 disappeared in Model 2 (i.e. after adjustment for the extra variables). These findings illustrate the polygenetic nature of CRAE and provide clues to the regions in which functional variants are likely to exist. The divergence of loci after adjustment for known coronary risk factors indicates the potential for interaction between important clinical and genotypic factors.

**LEFT VENTRICULAR HYPERTROPHY PREDICTS ADVERSE CARDIOVASCULAR EVENTS INDEPENDENT OF BLOOD PRESSURE IN TYPE 2 DIABETES MELLITUS**

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Type 2 diabetes mellitus (T2DM) is associated with left ventricular hypertrophy (LVH) and major adverse cardiovascular events (MACE). The clinical significance of concentric remodelling (CR) in the absence of LVH in T2DM remains controversial. We hypothesized that LVH and CR are common in subjects with T2DM and predict MACE. We studied 554 subjects with T2DM attending a complications surveillance program at Austin Health, Melbourne, Australia. LVH was defined as left ventricular mass indexed to height\(^3\) (LVMi\(^3\)); >49g/m\(^2\).7 in males and >45g/m\(^2\).7 in females, and CR as a relative wall thickness (RWT) >0.42 without LVH. MACE was defined as all cause mortality, stroke, heart failure admission, myocardial infarction or acute coronary syndrome and coronary and peripheral vessel revascularization. Follow-up was censored at first MACE at a mean of 4.9 ± 2.1 years. The mean ± SD age of subjects was 61.6 ± 12.7 years, mean body mass index (BMI) was 31.0 ± 5.9kg/m\(^2\) and 59% were male. During the follow up period, 150 MACE occurred. LVH was present on echocardiography in 56% of subjects, and CR in 17%. Those with LVH and CR were more likely to have MACE (Figure 1). With multivariate Cox regression analysis, LVH predicts MACE independent of age, gender, history of macrovascular disease, diabetes mellitus duration and blood pressure (HR 1.18; CI 1.04-2.39; P<0.05). We conclude that LVH is common in T2DM and predicts MACE.

**SERUM TOTAL CHOLESTEROL AUGMENTS AMBULATORY LEFT VENTRICULAR ARTERIAL LOAD: A MECHANISM OF END ORGAN DAMAGE?**

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Serum total cholesterol (TC) is purported to mediate end organ damage by mechanisms that are independent from brachial BP. However, TC is associated with increased arterial stiffness and we hypothesize this may impair ventricular-vascular interaction (VVI) and raise ambulatory central BP independent from brachial BP. In this study we examined the determinants of ambulatory central BP through comprehensive cardiovascular assessment to address this hypothesis. Healthy untreated men (n=27, aged 44±3 years, TC 5.3±0.2 [range 3.4-7.7] mmol/L) were tested at rest and whilst “ambulatory” (during activity at an intensity similar to daily activity). All participants underwent 24-hour ambulatory BP (24-ABP) monitoring and central BP assessment (radial tonometry). Combined tonometry and cardiorespiratory bioimpedance was used to assess left ventricular arterial load, determined by augmentation index (AIx) and effective arterial elastance index (Eai, end systolic pressure/stroke volume/m\(^2\)). AIx was defined as the ratio between left ventricular contractility (Eai, end-systolic pressure/endpoint-systolic volume) and arterial stiffness was assessed by carotid-femoral pulse wave velocity (PWV) and forearm microvascular blood flux (MBF) determined by laser Doppler velocimetry. From rest to ambulatory exercise there was a significant increase in brachial and central SBP, aortic PWV and MBF (P<0.001 for all). TC was related to ambulatory AIx (r=0.491, P<0.009, independent from 24-ABP and clinic brachial BP (r=0.695, P<0.001). Ambulatory AIx was also correlated to ambulatory Eai (r=0.593, P<0.001) and MBF (r=0.501, P<0.006). In multivariate analysis only age+TC (β=0.633, P<0.001) and ambulatory Eai (β=0.552, P=0.016) were independent predictors of ambulatory AIx (β=0.519; P<0.001). We conclude that raised TC adversely affects VVI and increases ambulatory LV arterial load, independent of brachial BP. This is a likely mechanism of TC-related end organ damage.

**EFFECTS OF EARLY INTENSIVE BLOOD PRESSURE LOWERING TREATMENT FOLLOWING ACUTE INTRACEREBRAL HAEMORRHAGE ON MIDLINE SHIFT AND HYDROCEPHALUS: THE INTERACT STUDY**

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The pilot phase of the Intensive Blood Pressure Reduction in Acute Cerebral Haemorrhage Trial (INTERACT) suggested that early intensive blood pressure (BP) lowering can attenuate haematoma growth at 24 hours and at 72 hours after intracerebral haemorrhage (ICH). The present analyses aimed to determine the effects of treatment on midline shift and hydrocephalus after acute ICH. The INTERACT study included 404 patients with CT-confirmed ICH.
elevated systolic BP (150 to 220 mmHg) and capacity to start BP lowering treatment within 6 hours of ICH onset. Patients were randomly assigned to intensive (target systolic BP, 140 mmHg) or standard guideline-based management of BP (target systolic BP, 180 mmHg) using routine intravenous agents. We randomly selected 100 patients from the INTERACT patients for the present analyses. CT scanning was done using standardized techniques with digital images analysed centrally. On baseline and 72 hour CT scans, midline shift volume (MSV) and Evans Ratio (ER) were measured. Study outcomes were the absolute and proportional increases in MSV and ER over 72 hours. In these 100 patients, mean systolic BP from 1 to 24 hours after randomisation was 17.7 mmHg lower in the intensive group than in the standard guideline group (P<0.001). Adjusted means of absolute and proportional increases in MSV were 0.3 mm and 6.2% in the guideline group and 0.8 mm and 10.6% in the intensive group, respectively. There were no significant differences between the two groups (difference in absolute increase, 0.5mm; 95% confidence interval (CI), –1.2 to 2.2 mm; P=0.56; difference in proportional increase, 4.4%; 95% CI, –9.1 to 17.6%; P=0.52). Similarly, adjusted means of absolute and proportional increases in ER were 0.001 and 0.5% in the guideline group and –0.003 and –1.0% in the intensive group, respectively, with no significant differences (difference in absolute increase, 0.004; 95% CI, –0.008 to 0.015; P=0.50; difference in proportional increase, 1.6%; 95% CI, –2.9 to 6.0%; P=0.49). In conclusion, early intensive BP lowering treatment after acute ICH has no significant effect on the development of midline shift or hydrocephalus.

### 30 PERIPHERAL AND CENTRAL AUGMENTATION INDEXES IN RELATION TO THE CYP4F2 POLYMORPHISMS IN CHINESE

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Background—Cytokine (CYP4F2) IFOS is a key metabolizing enzyme for the renal 25-hydroxyecosatrienoic acid (25-OH-ETA), which, as an endogenous vasokinin, may influence properties of the peripheral muscular arteries and arterioles. We, therefore, genotyped the CYP4F2 polymorphisms in relation to arterial wave reflections, as measured by augmentation indexes (Alx) in Chinese.

**Methods and Results**—We performed arterial measurements by SphygmoCor and genotyped three CYP4F2 polymorphisms (433M, rs9309398, and rs9309398) by PCR-RFLP in 1421 participants enrolled in the JinNing Population Study. A replication study for the V433M polymorphism was performed in 924 Chinese recruited from a workplace setting. Urinary 25-HETE concentration was determined by ELISA in a randomly selected subsample of 318 JinNing study participants. The CYP4F2 polymorphisms were genotyped in all JinNing participants, there was significant (Pc=0.02) interaction of the V433M polymorphism with sex and pulse rate in relation to peripheral and central Alx. M433 allele carriers, compared with V433V homoyzogotes, had significantly greater peripheral (5.0%, P=0.002) and central Alx (3.2%, P=0.001) in 693 men. The corresponding values were 2.7% (P=0.04) and 1.9% (P=0.04) in 490 subjects of the top tertile of pulse rate (≥76 beats/min), and were 4.0% (P=0.02) and 3.3% (P=0.02) in 315 replication participants with a pulse rate ≥76 beats/min. Urinary 25-HETE concentration was significantly higher (P=0.002) in M433M (0.98 ng/ml) than in V433V (0.83 ng/ml) subjects than in V433V homozygotes (2.06 ng/ml). There were no significant differences between the two groups (difference in absolute increase, 0.5ml; 95% confidence interval (CI), –1.2 to 2.2 ml; P=0.56; difference in proportional increase, 4.4%; 95% CI, –9.1 to 17.6%; P=0.52). Similarly, adjusted means of absolute and proportional increases in ER were 0.001 and 0.5% in the guideline group and –0.003 and –1.0% in the intensive group, respectively, with no significant differences (difference in absolute increase, 0.004; 95% CI, –0.008 to 0.015; P=0.50; difference in proportional increase, 1.6%; 95% CI, –2.9 to 6.0%; P=0.49). In conclusion, early intensive BP lowering treatment after acute ICH has no significant effect on the development of midline shift or hydrocephalus.

### 31 NEW ASPECTS TO THROMBOXANE A2 RECEPTOR BIOLOGY: OLD DOG, NEW TRICKS

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The eicosanoid thromboxane (TXA2) was first identified as a bioactive metabolite of arachidonic acid over 30 years ago. TXA2 acts in an autocrine/paracrine fashion to regulate cellular processes through binding to a cell surface receptor (TPα), a member of the G-protein linked receptor family. TXA2/TPα activation is a potent vasocostructor with prominent roles in initiating thrombosis and amplifying platelet aggregation. However, recent data suggests new pathological roles for this otherwise well characterized vasocostructor. TXA2 is a prominent regulator of inflammation and modulates reperfusion injury, affecting endothelial and myocyte survival. TXA2 is also implicated in tumour formation through both direct actions on endothelial and epithelial cells alike. Subsequent identification of the TP splice variant, TPβJ, has radically altered the perception of TPα in human disease. Many of the above effects are mediated specifically by the TPβJ isoform, which is only expressed in a highly restricted range of cells. The TPβJ isoform is anti-angiogenic, pro-tumorigenic and pro-inflammatory while the net effects of TPα activation are opposite to most of these actions. The majority of the novel effects of TPβJ are not related to heterotrimeric G-protein signaling; conversely, they are properties of the unique C-terminus of TPβJ which has multiple interactions domains not present in TPα. Indeed, we have discovered three proteins that interact with these residues in TPα which are required to manifest the anti-angiogenic activity of TXA2. Each of these interactions antagonizes a specific pro-angiogenic stimulus and when these interacting partners are absent then growth and mobility of cells are enhanced by TPα stimulation. These findings have re-defined the roles for TXA2, and its receptors in disease and, for the first time, have highlighted the (pathophysiological significance of the two TP isoforms in humans. This work is essential if we are to understand the human-specific basis for the regulation and treatment of disease. Beyond this, these findings open new frontiers in GPCR research, demonstrate the importance of understanding the splicosome and the role of non-traditional mediators in the regulation of health and disease.

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### 32 CHRONIC AT2 RECEPTOR STIMULATION IS ANTI-FIBROTIC IN AORTIC BANDED MICE

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The effects of AT2R on left ventricular hypertrophy are controversial in that increases, decreases or no change have been reported. On the other hand, AT2R stimulation is generally thought to cause an antioxidant response. However, all of the fore-mentioned cardiac AT2R effects rely on indirect inference from AT2R knock out studies. Therefore, the aim of this study was to determine the direct effects of chronic AT2R stimulation on cardiac remodelling induced by aortic banding. Male FVBn mice underwent either sham-operation or abdominal aortic banding for 4 weeks. During this time, mice received the AT2R selective agonist, CGP42112 (1µg/kg/min, sc via osmotic minipumps) alone or plus the AT2R antagonist PD123319. At the conclusion of the treatment period, mean arterial blood pressure (MAP) was determined, under isoflurane anaesthesia, via a direct aortic catheterisation, after which the hearts were removed for analysis of cardiovascular structure. Compared with the sham group (78:±3mmHg, n=6), banding significantly increased MAP (87:±2mmHg, n=6, P<0.05). CGP42112 did not have any effect on MAP in sham (n=6) or banded (n=7) groups. Similarly, banding increased ventricular body wall to heart ratio compared with sham (5.9:±0.9 vs. 3.5:±0.4), although these increases were unaffected by CGP42112. Cardiac left ventricular fibrosis was also measured in fixed hearts stained with Picrosirius Red. Banding (n=6) significantly increased left ventricular cardiac collagen content (5.2:±0.2% vs. sham 3.7:±0.2%, n=6, P<0.001). When sham-operated mice were treated with CGP42112 (n=6), there was no effect on left ventricular fibrosis. However, when banded mice were treated with CGP42112, left ventricular cardiac collagen content was markedly attenuated (3.7:±0.3%, n=6, P<0.001) to base line levels; effects that were reversed by co-administration of PD123319. Thus, for the first time, we have shown that direct chronic stimulation of the AT2R has striking anti-fibrotic effects on cardiac remodelling, confirming AT2R as a pharmacological target in cardiovascular disease.

### 33 AN INVESTIGATION OF THE NOVEL VASOPROTECTIVE ACTIONS OF NITROXYL (HNO)

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Cardiovascular disorders associated with endothelial dysfunction, like coronary artery disease and angina, have decreased endothelial nitric oxide (NO) bioavailability. However gynzcular troponin (GTPi), a NO donor, has not been examined for endothelial treatment in endothelial disorders, Corporate tolerance and reduced efficacy with long term use. With reports the reduced congener of NO, nitroxyl (HNO), relaxes rodent arteries without tolerance, this study aimed to assess HNO-mediated responses in human blood vessels to determine the therapeutic potential of HNO. Further, since the beneficial effects of NO include inhibition of leukocyte adhesion, the effect of HNO was assessed in this regard. Cumulative concentration-response curves (CRCs) to HNO were constructed in radial arteries.
These data show that in a model of hypertension induced by AII, exogenous H₂S treatment (NaHS, 10 mg/kg/day) significantly increased SBP, measured via tail-cuff method: untreated control 120 ± 3 mmHg, n = 9, AII-infused 171 ± 3 mmHg, n = 12, P < 0.001) and this increase in SBP was attenuated by treatment with NaHS (149 ± 7 mmHg, n = 11, P = 0.001) and was unaffected by inhibition of endogenous H₂S production (171 ± 5 mmHg, n = 11). Endothelial function was determined by maximum % vasoconstriction response to acetylcholine in aorta, measured by myography (control 82 ± 3%, n = 8), was attenuated in the AII-infusion group (59 ± 3%, n = 8, P < 0.01) and this attenuation was reversed by NaHS treatment (85 ± 4%, n = 5) and exacerbated by PPG treatment (51 ± 4%, n = 10, P < 0.001). Maximum relaxation to the endothelium independent vasorelaxant sodium nitroprusside was the same in all groups. Similarly, aortic superoxide anion production, by lucigenin-enhanced chemiluminescence (% control, n = 5), was significantly enhanced by AII-infusion (171 ± 18%, n = 11, P < 0.001), and this was reversed by NaHS treatment (123 ± 7%, n = 11, P < 0.05), but exacerbated by PPG treatment (181 ± 19%, n = 10, P < 0.001).

These data show that in a model of hypertension induced by AII, exogenous H₂S treatment in vivo reduces blood pressure, endothelial damage and vascular oxidative stress, whilst inhibiting endothelial NOS production in vivo is deleterious to these parameters. This further suggests that H₂S is a vasoprotective molecule that may be useful in the treatment of cardiovascular diseases.

ALIKSIEN INCREASES BRADYKININ LEVELS BY A MECHANISM UNRELATED TO RENIN INHIBITION OR TO INHIBITION OF BRADYKININ DEGRADATION

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Aliskiren is an orally active highly specific inhibitor of human renin, with IC₅₀ of 0.6 mmol/L for human renin, 4.5 mmol/L for mouse renin, and 80 mmol/L for rat renin. We previously reported that aliskiren (10 mg/kg/day) by subcutaneous osmotic minipump increased bradykinin levels in Ren-2 rats transgenic for mouse renin. To examine the relationship between increase in bradykinin levels and renin inhibition, we administered aliskiren by subcutaneous osmipump to Sprague Dawley rats, in which aliskiren has much lower potency for renin inhibition. Aliskiren at 10 mg/kg/day for 4 weeks increased bradykinin levels –2-fold in heart and lung, but not in blood, kidney, or brain; aliskiren also reduced Ang II and Ang I levels in kidney, but not in blood or in other tissues. A study of the time course of these responses showed aliskiren reduced renal Ang I levels within 24 hours, but a longer duration of treatment was required to increase bradykinin levels in heart and lung. A study of the dose-response showed that 4 weeks treatment with 3 mg/kg/day aliskiren increased cardiac bradykinin levels but did not influence renal Ang II or Ang I levels. To examine the effects of aliskiren on bradykinin degradation, we measured the acute hydrolysis response to intravenous bradykinin in rats administered 10 mg/kg aliskiren for 4 weeks; we found aliskiren did not potentiate the hydrolytic effects of bradykinin. We conclude that aliskiren increases bradykinin levels by a mechanism unrelated to renin inhibition or to inhibition of bradykinin degradation. In agreement with our previous studies of angiotensin converting enzyme inhibition and angiotensin receptor antagonism, renal angiotensin levels were the most sensitive indicator of renin inhibition. The increase in cardiac bradykinin levels during aliskiren administration may contribute to cardioprotection.

THE CONTRIBUTION OF INDOLEAMINE 2,3-DIOXYGENASE-1 TO THE REGULATION OF VASCULAR TONE IN EXPERIMENTAL ATHEROSCLEROSIS

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Objectives—Metabolism of tryptophan to kynurenine by indoleamine 2,3-dioxigenase-1 (IDO) is induced in systemic inflammation and contributes to the regulation of vascular tone. Therefore, we tested such function of IDO1 in atherosclerosis. Methods and Results—We generated ApoE−/− and IDO1 double knockout (ApoE−/−/IDO1−/−) mice. Following six months of Western diet, IDO1 was expressed in endothelial and other cells in lesions of ApoE−/− but not ApoE−/−/IDO1−/− mice, and its enzymatic activity increased as assessed by plasma concentrations of tryptophan and kynurenine. IDO1 was also expressed in endothelial cells of neo-veins in human atherosclerotic lesions. Addition of tryptophan released aortic rings of ApoE−/− mice, an effect blocked by the IDO1 inhibitor 1-methyl-D-tryptophan (1MT) and not observed with arteries of ApoE−/−/IDO1−/− mice. Administration of 1MT increased blood pressure in ApoE−/−/IDO1−/− mice but...
not Apoe1/– Apoe2/– mice, as assessed by tail-cuff method. Despite these activities, lesion sizes at different aortic sites were comparable in Apoe1/– Apoe2/– and Apoe1/– mice, except for the aortic arch in female mice where lesions were larger in Apoe1/– Apoe2/– mice. In addition, the content of collagen and macrophages in the lesion was increased in Apoe1/– Apoe2/– compared with Apoe1/– mice. Conclusions—Ido1 contributes to the regulation of vascular tone in experimental atherosclerosis.

39 SYMPATHETIC REGULATION DURING EARLY DEVELOPMENT OF OBESITY IN RABBITS

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Short-term consumption of a high fat diet (HFD) induces elevated blood pressure (BP), heart rate and renal sympathetic nerve activity (RSNA) which may be the basis for the development of long term obesity related hypertension1. In the present study we determined whether such changes reflected altered sympathetic regulation by examining the sympathetic responses to a range of stimuli including airjet stress, hypoxia and baroreflexes. New Zealand White rabbits implanted with telemetry devices for BP and RSNA were placed on a normal or 13.5 % HFD. Reflexes and stress responses were examined weekly during the 3 week diet. During this period, HFD rabbits increased BP, heart rate and RSNA by an average 6 %, 6 % and 50 % respectively (n = 9, P < 0.001) but there was no change in rabbits fed a control diet (n = 8). Acute airjet stress increased BP and RSNA which were blunted in HFD rabbits (over all weeks, BP –20 %, RSNA –70 %) but the tachycardia to airjet stress was not reduced until the 3rd week (~31 %). The sympathetic-excitatory responses to hypoxia were similar in the HFD and normal groups over the 3 week period of diet. HFD induced a reduction in the gain of the baroreflex because of an increase in lower plateau which indicates that higher basal levels of RSNA are being driven by a “non baroreceptor” mechanism. This effect is similar to the effects of the baroreflex on acute stress. Together these results suggest that sympathetic activation during a HFD is not a general facilitation of central sympathetic pathways but is it related to changes in baroreceptor or chemoreceptor afferent processing. However, the increased RSNA may be associated with a chronic activation of forebrain pathways mediating the sympathetic responses to acute emotional stress.1 Prior et al, Hypertension 2010; 55:862-868.

40 RAPID INCREASE IN BLOOD PRESSURE AND SYMPATHETIC ACTIVITY DURING CONSUMPTION OF A HIGH FAT DIET IN RABBITS

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Short term consumption of a high fat diet (HFD) by rabbits results in increased adiposity, blood pressure (BP), heart rate and renal sympathetic activity (RSNA). We have previously reported a relationship between plasma leptin concentrations and BP and RSNA after 3 weeks of fat feeding in these animals when both bodyweight and body fat are elevated. To better understand which factors drive the development of obesity hypertension, we examined the relationships between the dietary effects on body weight and plasma glucose and the cardiovascular and RSNA responses during the first 3 weeks of a HFD. New Zealand White rabbits implanted with telemetry devices for BP and RSNA were placed on a normal or 13.5 % F5. BP, heart rate and RSNA measured by radiotelemetry in the home cage rapidly increased by 4 %, 4 % and 11 % respectively (P < 0.05) as early as 1.5 days after starting a HFD. After 1 week on the HFD, rats achieved 4 %, 39 % and 30 % greater BP, heart rate and RSNA respectively (P < 0.05). By the end of 3 weeks of HFD, BP, heart rate and RSNA were elevated by 10 %, 5 % and 40 % respectively, compared to control (P < 0.05). The baroreflex gain was reduced by 11 % (P < 0.05) but there was no change in the baroreflex sensitivity (P > 0.05). At the end of the study, was 130 % higher in HFD compared to normal diet (n = 11). Intrathecal injection of angiotensin A (OX-A) (20 nmol) increased in mean arterial pressure (MAP), heart rate (HR), splanchnic sympathetic nerve activity (RSNA) and pheochromocytoma nerve activity (PNA). 5-nmole OX-A antagonist, SB 239670 (200 nmol), was unable to affect the resting level of cardiovascular parameters when injected intrathecally. On the other hand SB 334867, injected 20 min before OX-A (20 nmol), significantly reduced but not abolished the effects of intrathecal OX-A. Pressor response, tachycardia and sympathoexcitation intrathecal OX-A were attenuated by about 75 % when administered after SB 334867 and respiratory effects were reduced by about 50 %. These findings demonstrate; i) that the vascular sympathetic activity is mediated predominantly by the brainstem which it has been shown to be the fragment responsible for the central effects of CgA. The effects of vasostatin I (CgA17-76) (VS-I) delivered by intrathecal injection and direct microinjection into the RVL in cardio- pulmonary function in urethane anesthetized, vагotomised ventilated Sprague-Dawley rats (n = 21) were evaluated. The effects of intrathecal VS-I on somato-sympathetic, baroreceptor and peripheral chemoreceptor reflexes were also examined. At the concentration used (10, 100 or 200 μM for intrathecal or 5 μM for microinjection) VS-I produced no significant change in mean arterial pressure, heart rate, splanchnic sympathetic nerve activity, HR, splanchnic MAP or PNA nerve frequency. Somato-sympathetic, baroreceptor and peripheral chemoreceptor reflexes were unchanged following intrathecal VS-I. Our results indicate that vasostatin I is not the active proteolytic CgA fragment and may not be a central modulator of cardio-respiratory function and physiological reflexes.

42 BOTH OREXIN 1 AND 2 RECEPTORS MEDIATE OREXIN A INDUCED SYMPATHETOCOXITATION AND INCREASE IN PHRENIC NERVE ACTIVITY

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Orexin containing neurons in the lateral hypothalamus project to all levels of the spinal cord including dorsal horn, intermediolateral cell column and ventral horn. This study was undertaken to determine the role of orexin receptors in the spinal cord. Experiments were conducted under anaesthetized, vagotomised and artificially ventilated Sprague-Dawley rats (n = 17). Intrathecal injection of orexin A (OX-A) (20 nmol) caused increase in mean arterial pressure (MAP), heart rate (HR), splanchnic sympathetic nerve activity (RSNA) and pheochromocytoma nerve activity (PNA). But orexin receptor 1 antagonist, SB 239670 (200 nmol), was unable to affect the resting level of cardiovascular parameters when injected intrathecally. On the other hand SB 334867, injected 20 min before OX-A (20 nmol), significantly reduced but not abolished the effects of intrathecal OX-A. A pressor response, tachycardia and sympathoexcitation intrathecally OX-A were attenuated by about 75 % when administered after SB 334867 and respiratory effects were reduced by about 50 %. These findings demonstrate; i) that the vascular sympathetic activity is mediated predominantly by the brainstem which it has been shown to be the fragment responsible for the central effects of CgA. The effects of vasostatin I (CgA17-76) (VS-I) delivered by intrathecal injection and direct microinjection into the RVL in cardio- pulmonary function in urethane anesthetized, vагotomised ventilated Sprague-Dawley rats (n = 21) were evaluated. The effects of intrathecal VS-I on somato-sympathetic, baroreceptor and peripheral chemoreceptor reflexes were also examined. At the concentration used (10, 100 or 200 μM for intrathecal or 5 μM for microinjection) VS-I produced no significant change in mean arterial pressure, heart rate, splanchnic sympathetic nerve activity, HR, splanchnic MAP or PNA nerve frequency. Somato-sympathetic, baroreceptor and peripheral chemoreceptor reflexes were unchanged following intrathecal VS-I. Our results indicate that vasostatin I is not the active proteolytic CgA fragment and may not be a central modulator of cardio-respiratory function and physiological reflexes.
injection endothelial function was examined in mesenteric and femoral arteries. Endothelium-derived hyperpolarizing factor (EDHF)-mediated smooth muscle hyperpolarization and relaxation were halved in diabetes in mesenteric arteries. This was mediated by impaired activity of both intermediate (IK)- and small-conductance (SK) calcium-activated K⁺ channels (IK, SK channels). IK, SK channels, and NO bioavailability in mesenteric arteries was reduced, underpinned by upregu-
lation of NADPH oxidase isoforms. In contrast, endothelial function remained intact in femoral arteries in diabetes. Endothelial cell hyperpolarization due to activity of IK, SK channels was preserved. This hyperpolarization is unable to pass to the smooth muscle due to lack of myoendothelial gap junctions, therefore EDHF-mediated relaxation does not occur in this artery. The endothelium-dependent relaxation in femoral arteries is fully mediated by NO. Endothelial NO synthase expression and superoxide production were not altered in femoral arteries. This study demonstrates that the effects of diabetes on endothelial vasodilator dysfunction are region-dependent, with local mechanisms dictating functional outcomes.

**HYPERTENSION OF 3RD GENERATION – 3 FATTY ACID-DEFICIENT MICE**

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**Dietary** -3 fatty acid deficiency has been demonstrated to induce hypertension. However, the effects of multiple generations of -3 fatty acid deficiency remain unknown. In the present study, we examined the effects of 3 generations of deficiency with or without repletion in the final generation. In addition, we examined the hypothesis that increased blood pressure of -3 fatty acid deficient mice is due to eicosanoid production from an arachidonic acid (AA)-cyclo-
xoxygenase (COX) pathway. Male C57BL/6J mice were bred for three generations and fed diets either deficient (DEF) or sufficient (SUF) in -3 fatty acids. At postnatal day 21, the third generation offspring were kept on the dam’s diet or switched from dam’s diet to the opposite diet, creating four groups [SUF-SUF; DEF-DEF; SUF-DEF; DEF-SUF; n=15/group]. In addition, two subgroups that were treated with angiotensin 1-7 (Ang 1-7) 5 mmHg (DEF-DEF +, n=15/group; DEF-SUF +, n=15/group). At 25 weeks of age, systolic blood pressure was assessed by tail-cuff (CODA, Kent Scientific). Results obtained showed that DEF-DEF animals were hypertensive compared to SUF-SUF animals (108.7 ± 1.6 vs 96.5 ± 1.2 mmHg, P < 0.001). DEF-SUF animals were not hypertensive (98.4 ± 0.9 mmHg, ns vs SUF-SUF). SUF-DEF animals were hypertensive, but less so than the DEF-DEF animals (104.2 ± 1.5 mmHg; P < 0.05 vs SUF-SUF or DEF-DEF). In addition, treatment with the COX inhibitor prevented the hypertension of DEF-DEF animals (DEF-DEF +) 100.1 ± 1.9 mmHg vs SUF-SUF), but not the hypertension of DEF-DEF animals.

**CHRONIC ANGIOTENSIN 1-7 TREATMENT PREVENTS L-NAME-INDUCED HYPERTENSION AND CARDIOFIBROSIS INDEPENDENT OF MASR, AT_R, NITRIC OXIDE AND PROSTAGLANDINS IN ADULT MICE**

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We have previously reported that chronic treatment with Angiotensin 1-7 (Ang 1-7), reduces cardiac fibrosis in aged rats and mice. In these models, antifibrotic effects were inhibited by either PD123319 (angiotensin AT₁ receptor (AT₁R) antagonist) or AT779 (MasR antagonist), however, whether or not this effect was specific to the context of aging was not investigated. Therefore the aim of this study was to determine the cardiovascular effects of chronic Ang 1-7 treatment in adult (12-16 weeks) FVB/N mice, in which cardiac fibrosis was induced by 4 week nitric oxide synthase (NOS) inhibition (L-NAME 100mg/kg/day in drinking water). Systolic blood pressure (SBP) and cardiac fibrosis were significantly increased by L-NAME administration (FVB/N SBP: vehicle 83 ± 3 mmHg, L-NAME 114 ± 2mmHg, FVB/N cardiac collagen content: vehicle 2.4 ± 0.2%, L-NAME 7.0 ± 0.1%, n=10-11/group, P < 0.05). Ang 1-7 had no effect on blood pressure or cardiac fibrosis when given alone, but significantly inhibited detrimental effects of L-NAME when given simultaneously with NOS inhibition (FVB/N + L-NAME + Ang 1-7, SBP: 93 ± 3 mmHg; cardiac collagen content: 4.5 ± 0.3%, n=10, P < 0.05 vs FVB/N + L-NAME). Importantly, Ang 1-7 also inhibited L-NAME-induced hypertension and cardiac fibrosis in mice deficient in AT₁R (AT₁R KO, SBP: L-NAME 116 ± 6 mmHg, L-NAME + Ang 1-7 96 ± 5 mmHg; AT₁R KO cardiac collagen content: L-NAME 6.4 ± 0.6%; L-NAME + Ang 1-7 4.6 ± 0.3%, n=9-10/group, P < 0.05), suggesting that these effects were independent of AT₁R. Furthermore, blockade of pathways classically associated with Ang 1-7 signalling were also ineffective in reversing the effects of the Ang 1-7 treatment; AT779 (48mg/kg/r, s.c. via osmotic minipump), and indo methacin (indo, 2mg/kg/day in drinking water) did not reverse the blood pressure-lowering or antifibrotic effects of Ang 1-7 in AT₁R KO (SBP: L-NAME + Ang 1-7 + AT779 97 ± 3 mmHg, L-NAME + Ang 1-7 + indo 111 ± 5 mmHg; cardiac collagen content: L-NAME + Ang 1-7 + AT779 4.6 ± 0.3%; L-NAME + Ang 1-7 + indo, 4.3 ± 0.2%), suggesting that Ang 1-7 actions were independent of MasR, AT₁R, nitric oxide, and protaglandins. These results are in direct contrast to those previously determined in aged models, in which chronic Ang 1-7 effects were mediated by MasR and AT₁R. Thus this study emphasises the context specific nature of the protective effects of Ang 1-7, and highlights the importance of further investigation into the mechanisms of cardiovascular effects of Ang 1-7 in age-specific models of hypertension and remodelling.

**THE EFFECT OF ACUTE GREEN TEA SUPPLEMENTATION ON VASCULAR FUNCTION IN YOUNG OVERWEIGHT MALES**

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It is well-established that overweight individuals typically possess abnormal vascular function such as reduced forearm blood flow and high arterial stiffness. The purpose of this study was to examine the effects of acute supplementation of green tea containing epigallocatechin gallate (EGCG) on vascular function in young overweight males. Fifteen young males, with normal BMI profile, body mass index of 29.3 ± 0.4 (mean ± SEM) aged 35 years, participated in seven subjects. Augmentation index (AIx), a measure of arterial stiffness, was assessed using applanation tonometry and was calculated as the ratio of augmented pressure and pulse wave velocity.
pressure. Forearm blood flow (FFB), peak FBF, and forearm venous capacitance (FVC) were assessed using strain gauge Plethysmography with the venous occlusion technique. Blood pressure was monitored continuously using a beat-by-beat tonometry blood pressure sensor (Jentow, Colin Medical). Forearm vascular resistance (FVR) was calculated by dividing the mean arterial pressure (MAP) by FBF. During measurement, all subjects were seated at rest in a seated recline position. All variables were measured at baseline, 20, 40, 60, 80, 100, and 120 minutes following ingestion of green tea capsules contained 375 mg of EGCG or placebo. The order of each treatment was randomized, double-blinded with a one week wash-out period. There were no significant differences in FBF and AIx at baseline as well as FVR, MAP, and FVC across the time measured. However, FBF increased significantly (Fig. 1) by 46% at 120 minutes compared to placebo (P<0.02). AIx was decreased significantly (Fig. 2) by 73% at 120 minutes compared to placebo (P<0.045). The major finding was that acute ingestion of green tea capsules contained 375 mg of EGCG elevated FBF and reduced AIx in young overweight males.

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MAINTAINING BLOOD PRESSURE MEASUREMENT SKILLS IN A HEALTH SERVICE: AN INITIATIVE SUPPORTED BY THE HIGH BLOOD PRESSURE COUNCIL OF AUSTRALIA

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Increasingly busy clinical environments may lead to procedures for accurately measuring blood pressure (BP) being overlooked or ignored. The aims of our program were to standardize procedures and equipment for BP measurement across a major teaching and referral health facility. We also evaluated whether practical skills training introduced early in the Medical student program are retained if there is no later refresher training. We tested 107 monitors according to AS EN 1060.2-2002 for pressure scale accuracy and noted types of cuff sizes with each monitor. We found 14/56 (25%) of cuff sizes had both large and standard arm cuffs. Skills knowledge was assessed for Medical students (33 first year and 41 second year) and 166 clinical staff. Fig. 1 summarises the responses among the 3 groups to one of the questions, indicating revision of training is required, which has led to us implement refresher training. Our program increased staff enthusiasm and knowledge and can be transferred across other teaching and health services, to encourage best practice for measurement of blood pressure.

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CONTROL OF HYPERTENSION IS POOR IN 10-YEAR SURVIVORS OF STROKE

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Use of blood pressure (BP) lowering medications after stroke is important for reducing the risk of recurrent stroke. We aimed to determine whether BP lowering medications were being used as a secondary preventive therapy in survivors of stroke, and to determine the factors associated with BP control. We used the Australian Stroke Haemorrhagic Stroke Incidence Study to interview 1070 survivors of first-ever or recurrent stroke, excluding subarachnoid haemorrhage, from the North East Melbourne Stroke Incidence Study were interviewed at 10 years post-stroke. Individuals were classified as normotensive, controlled hypertension, uncontrolled hypertension, or uncontrolled hypertensive based on their measured BP level (cut-point 140/90 mmHg), past history of hypertension, and use of antihypertensive medications. At 10 years post-stroke, 371 (23.3%) of 1589 cases were alive. Of these, 299 (81%) had complete data on BP, antihypertensive medication use, and history of hypertension. Those with complete data were 5 years older than those without. A total of 76% of survivors were being treated with BP lowering medications as recommended in guidelines for stroke management. Eighty-five percent were hypertensive; 58% had controlled hypertension, 26% had uncontrolled hypertension, and 16% were unaware that they were hypertensive. Patients who did not know they had hypertension less often visited their General Practitioner than patients with controlled hypertension (Relative Risk Reduction 0.15, 95% Confidence Interval 0.04 to 0.60). Considerable improvement can be made to both the control of hypertension after stroke and in adhering to guidelines for stroke management.

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ABORIGINAL’S HEALTH AND NUTRITIONAL IMPROVEMENT FOR THEIR RISK REDUCTION

Marjorie Thorpe1, Mari Morii2, Atsumi Hamada2, Takashi Taguchi2, Hideki Morii2, Geoff Clark3, Violet Clark4, Yukio Yamori5, on behalf of WHO-CARDIAC Study group

Objectives: WHO-coordinated CARDIAC (Cardiovascular Diseases and Alimentary Comparison) study in 61 populations of 25 countries, has revealed the risks of the lifestyle-related diseases are increasing remarkably in Aboriginal people particularly, the present studies were designed to estimate their cardiovascular disease (CVD) risks in urban and rural Aboriginals compared with CARDIAC Study populations in the world and to reduce the risks by a dietary intervention. Methods: The first health examination in cooperation with Victorian Aboriginal Health Service, 2004, for a limited number of 107 male and female Aboriginal volunteers aged 20-74, living in Melbourne, and the second health survey in 2009 for 84 male and female Aboriginals living in rural Framingham were carried out by a simplified protocol of WHO-CARDIAC Study including anthropometrical and automated blood pressure measurements, blood and urine tests and questionnaires on diets and life styles. Further, for aiming the CVD risk reduction, after obtaining ethical approval from VHAS committee and informed consent from the participants, a dietary intervention study was carried out to offer two types of breads (soy bread containing 25g soy protein a day and regular wheat bread) for 2 months to the randomized 2 groups of Aboriginal volunteers, 33 in total. Findings: CVD risks such as obesity, hypertension, and dyslipidemia were remarkably increased in both urban and rural Aboriginals, compared with the average of 61 WHO-CARDIAC study populations in the world. Dietary intervention study showed significant improvements of body fat mass, blood pressure and atherogenic index calculated as non-high density lipoprotein/HDL, in the urban Aboriginal group eating soy protein bread compared with the placebo group. Conclusion: Health examinations of the urban and rural Aboriginal people confirmed significantly higher risks in Aboriginals, in comparison with all other CARDIAC Study populations in the world, even in whom Asian common dietary custom to eat soy beans daily was proven to be effective for CVD risk reduction, thus to hopefully contribute to Aboriginal health promotion.

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MICROALBUMINURIA IN RELATION TO THE METABOLIC SYNDROME AND ITS COMPONENTS IN A CHINESE POPULATION

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BACKGROUND: We investigated the prevalence of microalbuminuria and its association with the metabolic syndrome and its components in a Chinese population.

METHODS: The study subjects were recruited from a newly established residential area in the suburb of Shanghai. We measured anthropometry, blood pressure (BP), fasting plasma glucose, and serum lipids, and collected spot urine samples for the determination of albumin-creatinine ratio. We defined microalbuminuria as a urinary albumin-to-creatinine ratio from 17 to 299 mg/g in men and from 25 to 299 mg/g in women. The metabolic syndrome was defined according to the Adult Treatment Panel III criteria.

RESULTS: The 1079 participants included 410 (38.0%) hypertensive patients, and 66 (6.1%) diabetic patients. The prevalence of microalbuminuria (6.3%) was 2.6 times higher in 98 (9.1%) patients with the metabolic syndrome than 981 subjects with 2 components or fewer (14.3 % vs. 5.5%, P = 0.0007). In multiple regression adjusted for sex, age, body mass index, current smoking, and the use of antihypertensive medications, and mutually adjusted for the components, microalbuminuria was significantly associated with diastolic BP (odds ratio 1.60 for +10 mmHg; 95% confidence interval [CI] 1.08-2.36; P = 0.02)
and fasting plasma glucose (1.17; 95% CI 1.01–1.36; P = 0.04), but not (P = 0.10) with waist circumference, systolic BP, or serum HDL cholesterol and triglycerides.

**CONCLUSIONS:** Microalbuminuria is common in the Chinese population, and much more prevalent in the presence of the metabolic syndrome, mainly attributable to elevated diastolic BP and plasma glucose.

**PREVENTION OF DIABETES AND REDUCTION IN MAJOR CARDIOVASCULAR EVENTS IN STUDIES OF SUBJECTS WITH IMPAIRED GLUCOSE TOLERANCE: META-ANALYSIS OF RANDOMIZED CONTROLLED CLINICAL TRIALS**

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**Background:** Impaired glucose tolerance (IGT) is a pre-diabetic state, treatment of which may prevent or delay the onset of overt diabetes and thus potentially reduce major cardiovascular (CV) events. We therefore sought to determine whether interventions (including diet, exercise and pharmacological therapy), altered all-cause and cardiovascular related mortality in such subjects.

**Methods:** We performed a meta-analysis of prospective, randomised controlled trials (RCTs) that were identified in medical literature and databases. Trials were eligible for inclusion if they reported all-cause mortality rates (at a minimum), recruited approximately 100 patients and had a follow up of approximately one year. Interventions were divided into pharmacological and non-pharmacological.

**Results:** Ten RCTs that enrolled 23,152 patients met the above entry criteria. Diabetes was delayed or prevented by these interventions (risk ratio 0.83, 95% CI 0.80–0.86) vs control. Non-drug approaches (n = 3,495) were superior to drug-based approaches (n = 20,672) in diabetes prevention (0.52, 0.46–0.58 vs. 0.87, 0.84–0.89, P < 0.05). There was no difference in risk of all-cause mortality in the intervention versus control group (0.86, 0.84–1.10). There was also no difference in all-cause mortality when the drug sub-group only were considered (0.99, 0.85–1.15), nor in the non-drug sub-group (0.81, 0.61–1.09). There was also no difference in CV death (1.04, 0.81–1.78) or fatal and non-fatal myocardial infarction (0.59, 0.23–1.03) overall. Fatal and non-fatal stroke was, however, borderline reduced (0.76, 0.58–0.99) with intervention vs control.

**Conclusions:** Despite interventions being mostly successful in retarding progression to overt diabetes, this did not result in reduced all-cause mortality or death due to major cardiovascular events, with the possible exception of stroke.

**INFLATED CUFF PRESSURE SIGNIFICANTLY ALTERS PULSE WAVEFORM SHAPE ACQUIRED BY BRACHIAL CUFF VOLUME DISPLACEMENT TECHNIQUE.**

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Blood pressure waveform features and non-invasive central pressure estimation are increasingly used in research and clinical environments. Brachial cuff based biomedical devices often use an arbitrary absolute cuff pressure to measure waveforms. That is, cuff pressure is not relative to a person’s own blood pressure. This may alter the measured waveform shape, changing outcomes. This study investigates the impact of cuff pressure on pulse waveform shape. Supine brachial systolic, diastolic, and true mean blood pressure was measured in 14 healthy volunteers (age 25 to 60 years, 2 female) using the oscillogram technique. Brachial volume pressure pulse was measured using a standard brachial cuff inflated to: 10 mmHg below diastolic pressure (subdiastolic); mean pressure; 10 mmHg above systolic pressure (suprasystolic). At each cuff pressure, 10 seconds of data was acquired (sampling rate 2 kHz, 0.2 second Bartlett smoothing window) and averaged to form a single pulse waveform. Waveform shape was quantified in terms of form factor (meas–diastolic pressure)/pulse pressure, normalised root mean square (RMS), and frequency components. Waveforms acquired at each cuff pressure, the radial artery pressure waveform acquired by tonometry, and form factor calculated from brachial oscillogram blood pressure values were statistically compared. Form factor was significantly different (P < 0.001) between subdiastolic (0.56±0.04), mean pressure (0.49±0.05) and suprasystolic (0.24±0.03) cuff pressures. This was also significantly different (P < 0.001) to form factor acquired by oscillogram method (0.33±0.02) and radial tonometry (0.32±0.03). Oscillometric brachial and tonometric radial form factors were not significantly different (P > 0.05). Normalised RMS values did not differ between suprasystolic (0.41±0.04) and radial tonometry (0.42±0.03) but were significantly different (P < 0.001) between suprasystolic, mean pressure (0.59±0.03) and subdiastolic (0.64±0.03) values. Frequency decomposition confirmed the quantified waveform shape differences. Therefore, different inflated cuff pressures relative to a person’s own blood pressure significantly alter the recorded pulse waveform shape and the outcomes based on those waveforms.

**EFFECTS OF POSTURAL CHANGES ON AORTIC STIFFNESS ESTIMATED BY CAROTID-FEMORAL PULSE WAVE VELOCITY**

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Arterial stiffness (AS) is an independent factor of cardiovascular risk and is associated with mortality and morbidity. AS is conventionally assessed by carotid-femoral pulse wave velocity (PWVcf) in the supine position where the mean distending pressure is essentially uniform through the aortic trunk. This study investigates the effect of posture on AS where gravitational effects of the blood column produce a graded change in distending pressure, in addition to the mean arterial pressure (MAP). PWVcf and MAP were measured in 9 male subjects (age 25–37 yrs) during head-up tilt in 4 positions (0°, 30°, 65° and 90°). Transit time for PWVcf was obtained with applanation tonometry (SphygmoCor, Atcor Medical) and MAP was estimated by linear regression between BPr and FV (CCPr) and also between BPr and FV (CCPr). Rp was estimated by conventional methods (Rm) and also estimated with the effect of positive CCP on mean BPr and mean BPr with FV (Rp and Rpr, respectively). Cerebral arterial cross sectional compliance was estimated with an element windkessel model with and without consideration of positive CCP from BPr and from BPC.

**EFFECT OF PARENT VESSEL ON BLOOD FLOW IN ARTERIAL BIFURCATION ANEURYSMS**

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Saccular cerebral aneurysms occur mostly at the bifurcation of major arteries. The ability to explain aneurysm initiation and formation at the apex of bifurcations on morphological characteristics may be useful to predict cerebral aneurysms that are prone to rupture. The aim of this study is to elucidate the haemodynamic effects resulting from different aneurysm and parent artery morphology on intra-aneurysmal flow patterns and energy loss (EL). Computational fluid dynamic analysis was performed in an idealized middle cerebral artery bifurcation aneurysm model with various aspect ratios (aneurysm size / neck width) and parent artery diameters (2.34 mm) as a distinctive parameter. EL at the apex of the arterial bifurcation due to presence of the aneurysm was calculated as the energy difference between aneurysm inflow and outflow. Parent arteries of three different diameters were selected to investigate the role of the inlet boundary condition on intra-aneurysmal flow. For a similar range of aspect ratio (1.25–3.75), the mean EL for a 2 mm parent artery was 89.7 ± 1.4 % higher than for a 4 mm parent artery. Magnitude of maximum wall shear stress at the dome of the aneurysm reduces with increase in aspect ratio and parent artery diameter. The results show that for all parent arteries, increasing the aspect ratio leads to a reduced energy loss per aneurysm volume. Findings indicate that parent arteries morphology and diameter change the fluid dynamics of aneurysms and could be predictive of aneurysm rupture.

**NON-INVASIVE ESTIMATION OF CEREBRAL VASCULAR COMPLIANCE**

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Blood flow in cerebral vascular beds is highly regulated and depends on arterial storage capacity (arterial peripheral resistance Rp). Cerebral downstream critical closing pressure (CCP) is greater than zero. This positive CCP leads to an underestimation of Rp, which is conventionally estimated (Rm) as the ratio between mean arterial blood pressure (BP) and blood flow.

This study aims to compare the cerebral arterial cross-sectional compliance estimated from aortic BP (BPr) and from radial BP (BPr) accounting for the positive CCP. From 19 normal subjects (age 22 to 34 years, 9 female), BPr and cerebral flow velocity (Fv) from the middle cerebral artery were simultaneously recorded by means of applanation tonometry and Transcranial Doppler. BPr was estimated by use of a validated transfer function (SphygmoCor, Atcor Medical).

Cerebral CCP was estimated by linear regression between BPr and Fv (CCPr) and also between BPr and FV (CCPr). Rp was estimated by conventional methods (Rm) and also estimated with the effect of positive CCP on mean BPr and mean BPr with FV (Rpr and Rpr, respectively). Cerebral arterial cross sectional compliance was estimated with a two element windkessel model with and without consideration of positive CCP from BPr and from BPC.
Similarly, when lucigenin-enhanced chemiluminescence was employed to detect 359
superoxide radicals in rat isolated aortae, nitroxide or Nitrasartan (30 nM) displayed AT 1 receptor 360
antagonist activity. We report that the novel Nitrasartan, (30 nM) displayed AT1 receptor 361
antagonist combined with an antioxidant moiety (nitroxide) may therefore the aim of the present study was to use the native ligand Ang II as a template and 362
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to perform individual beta-aminergic acid substitutions and determine ATR binding selectively and functional vascular actions (in vitro AT2R-mediated vasorelaxation and in vivo vasodilator action). In competition binding experiments using either AT1R- or AT2R- transfected HEK293 cells, beta-tyrosine (Y)-Arg II and beta-isoleucine (I)-Arg II fully displaced iodinated Ang II from AT2R but not AT1R sites. In mouse aortic rings, beta-aminergic acid substitution at the Y and I residue of Arg II evoked vasorelaxation that was sensitive to blockade by the AT2R antagonist PD123319 and the NOS inhibitor L-NAME. In vivo influence of beta- Ang II peptides on blood pressure (BP) was determined in conscious male spontaneously hypertensive rats (SHR), either alone or in combination with AT1R and AT2R antagonists in a randomised treatment protocol, over several days in the same animals. When tested with a low level of AT1R blockade, beta-Arg II (15pmol/kg/min IV for 4 hours) reduced BP in conscious SHR (beta-Arg II + candesartan, −24 ± 4 mmHg) to a greater extent than candesartan alone (−11 ± 3 mmHg; n = 7, P < 0.05), an effect that was abolished by concomitant PD123319 infusion. However, in an identical experimental protocol, beta-Arg II had no influence on BP (n = 10). This current study demonstrates that a single beta-aminergic acid substitution resulted in a compound that demonstrated both in vitro vasorelaxation and in vivo depressor activity via a characteristic AT2R-mediated signalling pathway. This approach to the design and synthesis of novel AT2R-selective peptidomimetics shows great potential to provide insight into AT2R function.

Morphometric assessment of vascular structure showed that rats, when treated early, had significantly less vascular hypertrophy by markedly altering wall structure and content. This study shows that effects of ACE inhibition on BP and arterial wall stiffness varies in different parts of the aorta. Effects of ACE inhibition on BP and arterial wall stiffness were non-existent when given later in life.

ELEVATION OF PLASMA ANGIOTENSIN II LEVELS PROMOTES VASCULAR HYPERTROPHY BY MARKEDLY ALTERING WALL STRUCTURE AND CONTENT. This study shows that effects of ACE inhibition on BP and arterial wall stiffness varies in different parts of the aorta. Effects of ACE inhibition on BP and arterial wall stiffness were non-existent when given later in life.

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ASSESSMENT OF RISK RELATED TO CENTRAL BLOOD PRESSURE SHOULD BE CONSIDERED IN CONJUNCTION WITH OUT-OF-CLINIC BRACHIAL BLOOD PRESSURE

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Elevated clinic central blood pressure (CP) derived from tonometry predicts mortality independently of clinic brachial BP. Whether central BP may be useful to differentiate BP control has never been examined. Since central BP is dependent on BP taken at the time of recording tonometry, we hypothesized that central BP could not differentiate normotension (NT) from masked hypertension (MH) or white coat (WC) from essential hypertension (EH). However, due to wide disparity in PP amplification, we also hypothesized that central BP would be useful for risk stratification when used in conjunction with out-of-clinic BP. We studied 201 otherwise healthy treated hypertensive patients (aged 62 ± 8 years). Central BP was estimated by radial tonometry (Sphygmocor 8.1) and brachial BP by digital device (Omron HEM-907), as well as 24 hour ambulatory BP monitoring (A&D, TM-2420). BP control (NT, MH, WC, EH) was defined according to guidelines. Elevated clinic CP and brachial PP (PP) were defined as ≥50 mmHg. From the study population, there were 67 (33%) patients with NT, 59 (20%) with MH, 31 (15%) with WC and 44 (22%) with EH. There were no significant differences for clinic BP or PP between patients with NT and MH, or between patients with WC and EH (P > 0.05 for all). Furthermore, no other central BP variables could help differentiate BP control (P > 0.05 for all). However, 41% of patients had elevated PP (≥50 mmHg) with normal PP (<50 mmHg) and these included patients from all classifications of BP control (NT n = 27, MH n = 23, WC n = 6 and EH n = 14). Only 25% of the population had elevated PAP and PP; and these comprised patients from all BP control categories. We conclude that central BP alone cannot delineate different categories of BP control. However, when used in conjunction with out-of-clinic BP measures, knowledge of central BP may result in significant reclassification of risk related to BP.

MONOMACHINE OXIDASE A (MAOA) IS ASSOCIATED WITH DEPRESSIVE SYMPTOM PRESSURE SCORES AND BLOOD PRESSURE IN ADOLESCENT BOYS BUT NOT GIRLS

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Monomachine oxidase A (MAOA), a key enzyme that deaminates noradrenaline, adrenaline, serotonin, and dopamine, has been associated with mood disorders. A systolic blood pressure (SBP) quantitative trait loci has been identified in the region Xp11.4-Xq11 that contains MAOA. We hypothesised that single nucleotide polymorphisms (SNPs) within the MAOA gene would partly underlie the inverse association between depressive symptom scores and SBP we have detected in a population-based cohort of adolescents. This study analysed data collected on children at the year 14 follow-up (n = 974) from the Western Australian Pregnancy Cohort (Raine) Study. Tagged SNPs for MAOA were identified from Hapmap Phase II (CEU) data and genotyped. SBP was assessed by a trained assessor using a Dinamap electronic blood pressure recorder and depressive symptom scores were obtained from the Beck Depression Inventory for Youth (BDI-Y). A linear regression model was used to examine cross sectional associations between SBP (outcome) and tagged SNPs within MAOA. A mixed distribution model was used to account for the extra zero counts in our data and examine associations between depressive symptom scores and SBP and MAOA. After correcting for multiple testing, SNP RS90505859 within MAOA was independently associated with both SBP and depressive symptoms in adolescence boys but not girls. Boys with the CC (34.6%) genotype of RS90505859 compared to boys with the AC or AA genotype had higher SBP (115.0 mm/Hg vs. 112.4 mm/Hg; P = 0.004) and lower depressive symptom scores (1.7 vs. 1.9; P = 0.0004). These associations persisted after adjusting for depressive symptom scores and SBP respectively. We have shown for the first time that SNPs within MAOA may explain, in part, the co-occurrence of depressive mood and lower SBP in adolescent boys within the Raine Study. Additional cohorts may be needed to replicate and further explore this association.

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EFFECTS OF EARLY TREATMENT WITH ANGIOTENSIN CONVERTING ENZYME INHIBITION ON SEGMENTAL ISOBARIC AORTIC STIFFNESS AND WALL STRUCTURE IN ADULT SPONTANEOUSLY HYPERTENSIVE RATS

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Early angiotensin converting enzyme (ACE) inhibition in spontaneously hypertensive rats (SHR) during a critical treatment window prevents the development of hypertension in adulthood. However, the effects of such brief ACE inhibition on the structure and function of the aorta is still unknown. This study investigates the effects of ACE inhibition (perindopril, 3mg/kg/day, by gavage) in SHR (n = 6) treated at pre- (6-10 weeks) and established (20-24 weeks of age) hypertensive phases. Hemodynamic parameters were recorded in anaesthetised rats over a wide range of blood pressure (BP) induced by phenylephrine and sodium nitroprusside 1 week after withdrawal of treatment. Vascular stiffness of the thoracic and abdominal aortic segments was calculated from beat-to-beat pulse wave velocity (PWV) measurements in vivo, by using 1.4F pressure catheters inserted in the carotid and femoral arteries and positioned at a known distance apart. Histological and morphological parameters of the segmental aortic media were measured. Heart rate remained steady in all treatment groups. Perindopril significantly decreased systolic BP measured using a tail-cuff method (−32%, P = 0.0001), anaesthetic mean arterial BP (−43%, P = 0.0005), proximal (−31%, P = 0.0009), distal pulse pressure (−27%, P = 0.003), isobaric thoracic PWV (−19%, P = 0.002) but not abdominal PWV. All effects in BP and PWV were non-significant in SHR treated late. Morphometric assessment of vascular structure showed that rats, when treated early, significantly decreases medial cross sectional area (−40%, P = 0.0002), wall thickness (−24%), cross sectional area of the abdominal aorta but not 4%, ACE inhibition did not reduce the stiffness of the abdominal aorta despite concomitant decrease in both BP and vascular hypertrophy by markedly altering wall structure and content. This study shows that hypertrophy is not significant in the thoracic segment but stiffness was markedly reduced by ACE inhibition. This differential effect suggests the mechanism responsible for this change varies in different parts of the aorta. Effects of ACE inhibition on BP and arterial wall stiffness were non-existent when given later in life.

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EFFECTS OF PREHYPTERTENSION AND HYPERTENSION SUBTYPE ON CARDIOVASCULAR DISEASE IN THE ASIA-PACIFIC REGION

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Hypertension is traditionally divided into three main categories: isolated diastolic (systolic BP < 140 mmHg and diastolic BP > 90 mmHg), isolated systolic (systolic BP > 140 mmHg and diastolic BP < 90 mmHg) and systolic-diastolic hypertension (systolic BP > 140 mmHg and diastolic BP > 90 mmHg). While the clear evidence that isolated systolic and systolic-diastolic hypertension increase the risks of future vascular events, there remains an uncertainty about the risks of isolated diastolic hypertension. The objective of the study was to determine the effects of prehypertension and hypertension subtypes (isolated diastolic, isolated systolic and systolic-diastolic hypertension) on the risks of stroke and coronary heart disease in the Asia-Pacific region.
Pacific Region. The Asia Pacific Cohort Studies Collaboration was an individual participant data overview conducted by the principal investigators of cohort studies in the region. This analysis included 44 cohorts with a total of 587,171 participants. Outcomes included total (fatal and nonfatal) stroke and total (fatal and nonfatal) coronary heart disease. Stratified Cox proportional-hazards model was used to explore the relationship between CVD events and BP category (systolic age, cholesterol smoking and diabetes). Compared to normal BP (BP<120/80 mmHg), hazard ratios (95% confidence intervals) for total stroke were 1.64 (1.47-1.83) for prehypertension, 2.12 (1.79-2.51) for isolated diastolic hypertension, 2.86 (2.52-3.24) for isolated systolic hypertension and 4.82 (4.31-5.13) for systolic-diastolic hypertension. For coronary heart disease, hazard ratios (95% confidence intervals) were 1.29 (1.12-1.46) for prehypertension, 1.73 (1.43-2.10) for isolated diastolic hypertension, 1.72 (1.50-1.97) for isolated systolic hypertension and 2.43 (2.12-2.78) for systolic-diastolic hypertension. In subgroup analysis, larger hazard ratios of isolated diastolic hypertension were observed in Asia (2.46 in CHD and 2.35 in total stroke) compared with those in ANZ (1.47 in CHD and 1.36 in non-significant in total stroke). In Asia Pacific region, prehypertension and all hypertension subtypes were significantly associated with increased risks of stroke and coronary heart disease.

FACTORS ASSOCIATED WITH “IN-STUDY” BLOOD PRESSURE CONTROL: FINDINGS FROM THE 2 nd AUSTRALIAN NATIONAL BLOOD PRESSURE STUDY

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It is well-recognised that many people with elevated blood pressure taking antihypertensive therapy fail to achieve target levels following the commencement of drug therapy. The aim of the current analysis is to identify the baseline factors influencing “in-study” blood pressure control in elderly hypertensive subjects in ANBP2. In total, 6083 subjects were randomly allocated to antihypertensive therapy based on either an ACE inhibitor or Diuretic regimen. The results were compared with suggested strategies that emerged from the initial study. We found that GPs want a standardised approach to both the measurement and interpretation of blood pressure (BP). They want consistent, valid readings taken on one standardised, reliable device which accurately measures the patients’ BP in the ‘real world’. GPs expressed uncertainty about how to recognise the best current technology to measure BP, how multiple differing readings should be interpreted, and the “white coat” phenomenon. GPs want to be upskilled in areas such as the interpretation of ambulatory BP results, and the use of the cardiovascular risk assessment tool, and they want this information to be readily available through a single website, accessible via practice software. Suggestions were made for increasing patient awareness of BP and cardiovascular risk at both a population and individual level. High-quality preventive work is difficult to achieve in a time-poor environment with complex patients and proposals were made for improving this. Many suggestions were made for Medicare and funding changes to increase the uptake of home and ambulatory monitoring, and involving a third party to assist in the provision of individualised patient education. Reflective discussions arose regarding the patient-centred versus paternalistic approaches to patient management. The findings indicate that multiple, practical short and long-term approaches are needed at individual and population levels to facilitate improvements in preventive cardiovascular risk factor management. For the future, seasoned GPs and patients, including targeted education, to reduce the treatment gap are warranted.

A QUALITATIVE STUDY TO EXPLORE IMPLEMENTATION OF HYPERTENSION MANAGEMENT GUIDELINES INTO GENERAL PRACTICE

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Earlier research by this research team identified and explored the barriers to initiating treatment and treating hypertension to target BP in an adequate manner. We identified a number of factors that work against best practice blood pressure management in primary care. Given these findings we have conducted a needs assessment of general practitioners (GPs) to identify and explore strategies to improve the management of hypertension in primary care. A qualitative focus group method was employed. Twenty-five GPs and GP registrars (4 focus groups) from the Southern region of Tasmania participated in this study. The focus groups were recorded, transcribed and common emerging themes were analysed by an iterative thematic process. The results were compared with suggested strategies that emerged from the initial study. We found that GPs want a standardised approach to both the measurement and interpretation of blood pressure (BP). They want consistent, valid readings taken on one standardised, reliable device which accurately measures the patients’ BP in the ‘real world’. GPs expressed uncertainty about how to recognise the best current technology to measure BP, how multiple differing readings should be interpreted, and the “white coat” phenomenon. GPs want to be upskilled in areas such as the interpretation of ambulatory BP results, and the use of the cardiovascular risk assessment tool, and they want this information to be readily available through a single website, accessible via practice software. Suggestions were made for increasing patient awareness of BP and cardiovascular risk at both a population and individual level. High-quality preventive work is difficult to achieve in a time-poor environment with complex patients and proposals were made for improving this. Many suggestions were made for Medicare and funding changes to increase the uptake of home and ambulatory monitoring, and involving a third party to assist in the provision of individualised patient education. Reflective discussions arose regarding the patient-centred versus paternalistic approaches to patient management. The findings indicate that multiple, practical short and long-term approaches are needed at individual and population levels to facilitate improvements in preventive cardiovascular risk factor management. For the future, seasoned GPs and patients, including targeted education, to reduce the treatment gap are warranted.

NADPH OXIDASE AND TGF-ß SIGNALING PROMOTE EXTRACELLULAR MATRIX PRODUCTION BY CARDIAC FIBROBLASTS

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Oxidative stress, especially that due to activation of the superoxide-generating, Nox-containing NADPH oxidase is involved in the development of cardiac fibrosis. By knocking down cardiac Nox4 gene expression, pressure-overload-induced cardiac fibrosis was reduced indicating that Nox4 is an important mediator of adverse fibrosis.1 We hypothesized that the profibrotic transforming growth factor-β (TGF-β) acts via Nox4 to mediate fibrotic responses in mouse cardiac fibroblasts. TGF-β1 (10 ng/mL; n = 3) stimulated gene expression of collagen I (by 21 ± 0.4 fold) and the fibroblast differentiation marker smooth muscle α-actin in mouse cardiac fibroblasts (by 2.8 ± 0.6 fold). Nox2 and Nox4 are the major isozymes of the NADPH oxidase catalytic subunit found in cardiac fibroblasts. TGF-β1 upregulated Nox4 gene expression (2.9 ± 0.8 fold) without affecting Nox2 gene expression.1,2 Whilst Nox1 was undetectable, EUK-134, a scavenger of superoxide and hydrogen peroxide, suppressed the TGF-β–induced collagen gene expression (by 46 ± 13%; n = 4). Furthermore, by expressing a dominant negative form of Nox4 in fibroblasts to suppress the oxidative activity, TGF-β–induced hydrogen peroxide production and collagen gene expression were suppressed (by 33 ± 1% and 60 ± 5% respectively), suggesting that Nox4-dependent ROS are important for TGF-β signaling in extracellular matrix production. The effect of sRNA.Tg against Nox4 gene expression on TGF-β–induced matrix synthesis is under investigation. In conclusion, Nox4 may act as an effector molecule in cardiac fibroblasts mediating fibrotic reactions, by acting as an intermediary in the signaling of TGF-β. Further work will define its role in adverse cardiac fibrosis in vivo.


ASSOCIATION OF ANGIOTENSIN CONVERTING ENZYME 2 GENE POLYMORPHISMS WITH BLOOD PRESSURE AND SYSTOLIC FUNCTION IN AUSTRALIAN SUBJECTS WITH TYPE 2 DIABETES

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The angiotensin converting enzyme 2 (ACE2) gene is located on chromosome Xp22 and expressed in cardiac and renal tissue. Diabetes activates the renin-angiotensin system (RAS) and polymorphisms are associated with systolic and diastolic blood pressure (BP) and cardiac abnormalities. Subjects with type 2 diabetes mellitus (T2DM) attending a complications clinic at the Royal Melbourne Hospital (n = 25) were prospectively analysed for LDH content. Basal left ventricular developed pressure (LVPD) was similar in all groups (mm Hg; WT vs T2DM: male: 70 ± 1 vs 70 ± 2; female: 70 ± 2 vs 77 ± 2, P = ns), but dp/dt min was attenuated in ArKO hearts (mm Hg/s; male: −323 ± 139 vs −224 ± 91; female −266 ± 120 to −244 ± 112, P = 0.05). Ischemic contracture was diminished in ArKO hearts (mm Hg; 49 ± 3 vs 40 ± 2, P < 0.05), consistent with an improved recovery of end diastolic pressure observed in perfused (mm Hg; 48 ± 8 vs 28 ± 3, P < 0.05). Recovery of LVPD was significantly increased in ArKO hearts and this differential was accentuated in females (40 ± 9 vs 35 ± 6%，female 62 ± 8 vs 30 ± 5%, P < 0.05). Improved functional recovery in ArKO hearts was associated with a significantly reduced LDI content of coronary effluent in perfusion (μg: male: 27.8 ± 10.0 vs 7.8 ± 1.1%; female 22.4 ± 7.9 vs 1.0 ± 3.4%, P < 0.05). These findings contrast with poor experimental outcomes associated with overactivity and suggest that cardioprotection from ischemia/reperfusion may be facilitated by a fundamental shift in the estrogen/testosterone balance. Further investigations are required to establish the molecular mechanisms involved.

CARDIAC DIMENSIONS ARE LARGELY DETERMINED BY DIETARY SALT IN PATIENTS WITH PRIMARY ALDOSTERONISM, BUT NOT IN PATIENTS WITH ESSENTIAL HYPERTENSION

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Animal studies have demonstrated that dietary sodium intake is a major influence in the pathogenesis of aldosterone-induced effects in the heart such as left ventricular (LV) hypertrophy and fibrosis. LV hypertrophy is an important predictor for cardiovascular morbidity and mortality. We investigated and compared relationships between (1) aldosterone and (2) dietary salt and LV dimensions in patients with either primary aldosteronism (PA) or essential hypertension (EHTN). Patients with confirmed PA (n = 56) or EHTN (n = 25) were prospectively analysed for echocardiography and diastolic function. LV cavity dimensions were similar between groups (mmHg; WT vs ArKO; male: 70 ± 1 vs 70 ± 2; female: 70 ± 2 vs 77 ± 2, P = ns). Isolated LV diastolic dysfunction was identified by EHTN in males (54 ± 3 ± 6%, female 62 ± 8 ± 30 ± 5%, P < 0.05). Improved functional recovery in ArKO hearts was associated with a significantly reduced LDI content of coronary effluent in perfusion (μg: male: 27.8 ± 10.0 vs 7.8 ± 1.1%; female 22.4 ± 7.9 vs 1.0 ± 3.4%, P < 0.05). These findings contrast with poor experimental outcomes associated with overactivity and suggest that cardioprotection from ischemia/reperfusion may be facilitated by a fundamental shift in the estrogen/testosterone balance. Further investigations are required to establish the molecular mechanisms involved.

ELEVATED LEPTIN LEVELS IN OBESE CONTRIBUTE TO THE DEVELOPMENT OF HYPERTENSION THROUGH ACTIONS IN THE DORSAL MEDIAL HYPOTHALAMUS

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Leptin, an adipose derived cytokine, is elevated in most forms of obesity; and is positively correlated with leptin levels in an obese mouse (DIO) model. To investigate whether leptin contributes to obesity induced hypertension, diet induced obese mice (DIO) (diet/leptinemic mice) were fed a high fat diet (45% fat) and lean control (LC). Leptin deficient mice were fed a standard fat diet (12% fat), p38AT, expression, a marker of leptin receptor signalling was measured by immunohistochomatic (IHC). Heart rate (HR) and BP were measured by radiotelemetry. DI (54.7 ± 0.7 g, n = 23) and obo (52.9 ± 1.7 g, n = 3) mice were both significantly heavier compared to lean mice (32.7 ± 0.6 g, n = 12, P < 0.001). Elevated mean arterial pressure (MAP) and HR was only observed in DIO mice (113.5 ± 1.5 mmHg, P < 0.001; 580 ± 1.7 mmHg, P < 0.001, n = 23) compared with lean mice (104.9 ± 1.6 mmHg; 504 ± 0.9 mmHg; n = 12). There was a positive relationship between plasma
leptin levels and MAP of mice (r^2=0.6, P<0.001). To inhibit the central actions of leptin, DIO mice were treated twice daily with a leptin antagonist given directly in the lateral ventricle. After 7 days of treatment MAP decreased from 118.5±6.6 to 111.3±5.1 mmHg (n=5, P<0.034) in DIO mice and was no longer significantly higher than lean mice (104.9±1.6 mmHg, n=12, P=0.128). pSTa3 expression was increased in the dorsol medial hypothalamic (DMH) in response to leptin in both lean and DIO mice (181.9±45.5, 247.6±48.8 pSTa3 positive cells, respectively) compared to vehicle treated lean and DIO mice (13.16±7.5; 59.2±20.9 pSTa3 positive cells, respectively, P<0.02). This indicates leptin resistance is selective to the arcuate region of the hypothalamus, with resistance not developing in all other hypothalamic regions in obesity including the DMH. These results demonstrate that leptin and not obesity plays a predominant role in elevating blood pressure. Furthermore, elevated leptin levels, with the ability to activate central receptors seems to be critical to induce obesity-associated hypertension. Selectively targeting actions of leptin in the brain in obesity, possibly the DMH has the potential for treating obesity-associated hypertension.

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NEUROGENIC HYPERTENSION IN YOUNG AND ADULT SLACGER HYPERTENSIVE MICE INVOLVES DIFFERENT GENES ACTING BY SIMILAR MECHANISMS

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The hypothalamus has an important etiological role in neurogenic hypertension. It also mediates stress responses in the Schlagar high blood pressure (BPH/BPZH) mouse, a genetic model of neurogenic hypertension. Here we identified the hypothalamic genes whose expression differs between BPH/2J and normal BP (BPH/2J) strains at both young (<8 week-old) and adult (25 week-old) ages. Hypothalamus was removed from male BPH/2J and BPZH mice (n=12 per age) at the peak of circadian BP. CDNA samples were applied to Affymetrix GeneChip2 Mouse Gene 1.0 ST Arrays. In young mice, we identified 1017 genes whose expression differed between the strains after false discovery rate (FDR) adjustment of 0.001. In adult mice, 466 genes were identified (FDR<0.05). The vast majority of these genes have not been implicated in hypertension previously, and the function of many is not known. At both ages we saw differences in expression of genes with known roles in inflammation and oxidative stress, and especially ones that decrease nitric oxide production and that cause mitochondrial dysfunction. These could result in increased production of reactive oxygen species that, via their signalling functions, could account for the alterations we saw in expression of certain other genes, including increases in genes for ribosomal proteins and transcription factors. Also of interest were changes in expression of genes for neuropeptide Y receptor Y2 (Npyr2) and pro-opiomelanocortin (Pocm) whose products can increase sympathetic activity in young BPH mice. The genes for hypocretin (Hcrt) and neuropeptide S receptor 1 (Npsr1), involved in sympathetic activity and response to stress, showed increased expression at both ages. These and other genes were validated by quantitative real-time PCR. Gene ontology and gene set enrichment analyses provided added support to these findings. Our study has assisted in the identification of possible mechanisms that cause and maintain hypertension in this neurogenic model. They reveal that similar pathways are involved at these different phases of the condition.

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BILATERAL VAGOTOMY DIFFERENTIALLY ALTERS THE HEART RATE AFTER ACUTE INTERMITTENT HYPOXIA

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Acute intermittent hypoxia (AIH) is a frequently studied experimental model of obstructive sleep apnoea (OSA), AH can elicit a long-term increase in sympathetic output (long-term facilitation, LFT). Most investigations study AH in mechanically ventilated, bilaterally vagotomised, and anaesthetised animals. Under these conditions, the effect of AH on the parasympathetic nervous system (e.g. heart rate) cannot be observed. We hypothesised that heart rate change after AH would also be regulated by LTF of vagus nerve activity. In urethane-anaesthetized, mechanically ventilated Sprague-Dawley rat, we investigated the effect of ten 45 s episodes of 10% O2-90% N2 on the heart rate and blood pressure change. We then tested whether or not hypoxic chemoreceptor reflex were changed 60 min after AIH. In bilaterally vagotomised animal hypoxic chemoreceptor reflex were changed 60 min after AIH. In bilaterally vagotomised animal hypoxic chemoreceptor reflex were changed 60 min after AIH. In bilaterally vagotomised animal hypoxic chemoreceptor reflex were changed 60 min after AIH. In bilaterally vagotomised animal hypoxic chemoreceptor reflex were changed 60 min after AIH. In bilaterally vagotomised animal hypoxic chemoreceptor reflex were changed 60 min after AIH. In bilaterally vagotomised animal hypoxic chemoreceptor reflex were changed 60 min after AIH. In bilaterally vagotomised animal hypoxic chemoreceptor reflex were changed 60 min after AIH. In bilaterally vagotomised animal hypoxic chemoreceptor reflex were changed 60 min after AIH. In bilaterally vagotomised animal hypoxic chemoreceptor reflex were changed 60 min after AIH. In bilaterally vagotomised animal hypoxic chemoreceptor reflex were changed.
ABPM remains a useful research tool to explore relationships between blood pressure and pregnancy outcomes; however its clinical role is best restricted to aiding an initial diagnosis of white coat or essential hypertension in the first half of pregnancy, thereby permitting an appropriate plan for subsequent clinical management and selective use of antihypertensives in such women.

**NOVEL PRACTICAL APPLICATION OF HOME BLOOD PRESSURE MEASUREMENTS SUPERIORITY TO AMBULATORY BLOOD PRESSURE MONITORING AND APPLICATION IN PREGNANT WOMEN**

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Clinic blood pressure (CBP) measurements is still a gold standard for diagnosis and treatment of hypertension. However, a significance of CBP has been questioned and a clinical significance of home blood pressure (HBP) measurements and ambulatory (ABP) monitoring is drawing attention. HBP and ABP are characterized by increased measurement frequency and increased information of blood pressure. ABP are characterized by increased measurement frequency and increased information of blood pressure in relation to time. We have monitored the population of Ohasama, with respect to their prognosis and have reported that HBP is superior to CBP in the prediction of CVD morbidity and mortality. The risk stratification system proposed in the ESH-ESC guidelines has a stronger predictive power using HBP-based classification compared with CBP-based one. We examined the prognostic significance of white-coat (WC) HT for CVD and found that the relative hazard in patients with WCHT was significantly lower than that for true HT but observed that the development of sustained HT was more frequent in patients with WC HT than those with true normotension during 10-year observation period. Poor prognosis of masked HT has also been confirmed. Masked HT is one of the phenotypes of morning HT, which is mediated by disturbed circadian BP variation and/or insufficient duration of action of drugs. Ohasama study also demonstrated that non-dippers and risers have a poor prognosis. Recently we developed new devices for HBP which can automatically monitor nocturnal BP during sleep. Such characteristics of modern HBP can assess BP control during treatment including a duration of action. The Ohasama study also demonstrated that increases in day-by-day variability of morning HBP as well as short-term BP variability based on ABP were associated with an increase in the risk of CVD mortality. HBP measurements improve the accuracy of screening for HT, assess of BP control during treatment, encourage drug compliance and have a beneficial effect on the medical economics. On the basis of these results, I will discuss which measurements, CBP, HBP and ABP is superior in the practice. Furthermore I will state the importance of HBP in a special reference to the application of HBP in pregnant women.

In conclusion, HBP should be measured for modern diagnosis and treatment of HT and in the near future HBP is sure to replace CBP in the diagnosis and management of hypertension.

**IS BLOOD PRESSURE TOO VARIABLE TO MEASURE?**

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Assessments of blood pressure are among the most common measurements made in clinical practice. While it is widely recognized that an individual’s blood pressure may vary from moment to moment, the clinical implications of this background variability are not well understood by most practitioners. In particular, the impact of ‘noise’ on the validity of clinical decision making is widely underestimated. The diagnosis of hypertension requires that blood pressure is above or below a given cut point and this requires a test that is both sensitive and specific. The clinical assessment of blood pressure will only meet these criteria if the number of blood pressure measurements recorded is very large - more often than not in clinical practice only a few measurement of blood pressure are made and only a rudimentary assessment of the true underlying blood pressure level of an individual is achieved. Once a decision to commence antihypertensive drug treatment is made, patients typically enter a period of short- and then long-term monitoring to determine the effectiveness of the treatment. Here again the ‘noise’ in blood pressure measurement presents significant challenges. The usual effect of a drug is to lower blood pressure by about 5-10mmHg and less for titration of doses. Changes of this magnitude in an individual can only be detected against usual background variability if many tens of blood pressure measurements are made before and after. Which again is not usual clinical practice. Fortunately, from analyses of datasets from large-scale trials of blood pressure lowering, we see that the apparent large variability in response of individual’s to drug therapies is much less than is perceived. What appears to be variation in response between individuals is mostly just a consequence of measurement error caused by the background variability (noise) and not true differences in response. As such, the difficulty of detecting the blood pressure response to the commencement of treatment becomes less of an issue because, despite appearances to the contrary, most people actually respond in much the same way. With the need to precisely diagnose the presence or absence of hypertension according to a given cut point being concurrently obviated by the introduction of absolute risk assessment techniques the need for a precise measurement of blood pressure in the clinical setting is set to decline. There is an urgent need for these observations to be incorporated into evidence-based guidelines for the monitoring of blood pressure, to ensure that decision making is fully informed, scarce healthcare resources are efficiently applied and clinical actions are as informed as possible.