Abstracts From the
36th Annual
Scientific Meeting of
the High Blood Pressure Research
Council of Australia

Adelaide, Australia
November 26-28, 2014
Editor: Brian J. Morris

Publication supported by

SERVIER
Improving lives through discovery
CONTRIBUTION OF THE AREA POSTREMA TO THE INCREASED CARDIAC SYMPATHETIC NERVE ACTIVITY IN OVINE HEART FAILURE

Abukar Y, Ramchandra R, May CN

The Florey Institute of Neuroscience and Mental Health, Melbourne, Victoria, Australia

Background: Heart rate (HR) is associated with an increase in cardiac sympathetic nerve activity (CSNA), which is directly linked to mortality in HF patients. The mechanisms responsible for the elevated CSNA remain unclear. Previous studies indicate that the area postrema (AP), a circumventricular organ in the brainstem, plays a role in the control of sympathetic nerve activity. We hypothesized that the elevated CSNA in HF is mediated by the AP and lesioning this region would reduce the increased CSNA in shear with HF.

Aims: To determine the effect of sham lesion or lesion of the AP on CSNA and hemodynamics in conscious sheep with HF.

Methods: Studies were conducted in 2 groups of sheep with pacing-induced HF: sham (n=6) and AP lesion (n=6) sheep. Mean arterial blood pressure (MAP), heart rate (HR) and CSNA were recorded simultaneously in conscious sheep at least 4 days after surgery.

Results: Heart rate was associated with a significant decrease in ejection fraction (from 74±2 % to 38±1 %; P<0.001), which was similar in both groups. There was a significant reduction in CSNA burst incidence in the AP lesion group compared with the sham group (45±10 and 89±3 bursts/100 heartbeats, respectively; P<0.01).

Conclusions: In sheep with HF, the group with lesion of the AP had a significantly lower CSNA compared with the sham group. These data suggest that the AP plays a role in setting the detrimental high levels of CSNA in HF.

G PROTEIN-COUPLED ESTROGEN RECEPTOR SIGNALING IMPROVES STROKE OUTCOME IN FEMALE MICE

Broughton BRS, Jansen GL, Sobey CG

Department of Pharmacology, Monash University, Clayton, Victoria, Australia

Background: Estrogen has been assumed to provide neuroprotection following stroke entirely via classical estrogen receptors. Interestingly, there is recent evidence that activation of a novel G protein-coupled estrogen receptor (GPER) with the selective ligand G-1 can improve stroke outcome in ovarectomized mice. However, it remains to be determined if the neuroprotection provided by endogenous estrogen occurs via GPER signaling.

Aims: To test if the selective GPER antagonist G-15 worsens stroke outcome and to examine whether tamoxifen, a clinically approved GPER agonist, provides neuroprotection post-stroke.

Methods: To address the first aim, intact female mice C57Bl6 mice were treated i.p. with G-15 (300 µg/kg, n=7) or vehicle (dimethyl sulfoxide, n=8) 1 h prior to 0.5 h middle cerebral artery occlusion (MCAO). To address the second aim, ovariectomized mice were treated i.p. with tamoxifen (10 µg/kg, n=8), vehicle (dimethyl sulfoxide, n=7), or a combination of tamoxifen and G-15 (n=5) 1 h prior to MCAO. In addition, T cell or neutrophil infiltration into the ischemic hemisphere was examined using CD3 or myeloperoxidase immunohistochemistry, respectively.

Results: After 24 h, intact female mice treated with G-15 showed worsened functional outcomes and increased infarct damage compared with vehicle. Furthermore, immunohistochemistry showed a significant increase in neutrophils, but not T lymphocytes in the ischemic hemisphere of G-15-treated mice. Ttamoxifen-treated mice had significantly improved functional outcomes and ~60% smaller infarct volume (P=0.05) compared to vehicle. The neuroprotective effects of tamoxifen were blocked in the presence of G-15.

Conclusions: These results suggest that in females, GPER activation contributes to estrogen-mediated neuroprotection following stroke, and that tamoxifen can improve stroke outcome following surgical menopause.

EFFECTS OF ANTI-HYPERTENSIVE TREATMENT ON FUNCTIONAL AND STRUCTURAL COMPONENTS OF LARGE ARTERY STIFFNESS AND RETINAL VESSEL DIAMETERS IN A RODENT MODEL OF TYPE 1 DIABETES


Australian School of Advanced Medicine, Macquarie University, Sydney, New South Wales, Australia; Departmnet of Cardiology, University of Tartu, Estonia

Background: Diabetes is associated with cardiovascular risk, increased arterial stiffness, and ocular diseases. Whether large artery stiffness is independently associated with diabetes per se or concomitant hypertension is currently unknown.

Aims: To determine whether artery stiffness is independently associated with diabetes or is associated concomitantly with hypertension.

Methods: Male, Wistar rats (6 weeks of age) were divided into control (n=8), control with anti-hypertensive treatment (telmisartan, 10 mg/kg/day, n=8), induced diabetes (intraperitoneal streptozotocin, 50 mg/kg, confirmed by blood glucose measurement, n=12) and diabetes with anti-hypertensive treatment (n=12). At 18 weeks, rats were anaesthetized (urethane, 1.3 g/kg) and aortic pulse wave velocity (aPWV, aortic stiffness) was measured invasively across a full range of physiological arterial pressure (infrarenal phenylephrine, sodium nitroprusside, 30 µg/kg/min). Retinal artery and venous diameters were measured using a custom microscope/camera assembly. Passive (elastin, collagen) and active (endothelial, smooth muscle function) components of arterial vessel stiffness were quantified using tensile testing and myography.

Results: Conscious, systolic blood pressure was high in both control and diabetic animals (142±16 and 132±22 mmHg, respectively) compared to control and diabetic animals on anti-hypertensive therapy (105±11 and 119±14 mmHg; P<0.01). Diabetic animals had marginally but significantly lower aPWV across all pressures. Anti-hypertensive treatment decreased aPWV within the low pressure range and increased aPWV within the high pressure range for both controls and diabetic animals. This resulted in increased pressure dependency of aPWV with anti-hypertensive treatment. Retinal venous diameters were greater with diabetes. Anti-hypertensive therapy increased retinal venous diameters but decreased arterial diameters. There was no difference in aortic endothelial dependent or independent vasorelaxation. Sensitivity to phenylephrine (vasoconstriction) was less in diabetic animals (P<0.05). Anti-hypertensive therapy caused a rightward shift in the aortic stress-strain curve (P<0.001).

Conclusions: Diabetes appeared to have a small but positive effect on arterial stiffness when studied independently of blood pressure. However, high blood pressure decreased the artery’s ability to respond to acute pressure changes, possibly due to remodelling of passive arterial wall components. Retinal venous diameters were greater with diabetes, with anti-hypertensive therapy having different effects on the retinal arteries and veins.

PREDICTION OF HEART FAILURE BY SERUM AMINO-TERMINAL-PRO-B-TYPE NATRIURETIC PEPTIDE (NT-proBNP): AN INTERIM ANALYSIS OF THE SCREENING EVALUATION OF THE EVOLUTION OF NEW HEART FAILURE (SCREEN-HF) STUDY


*St. Vincent's Institute of Medical Research, Melbourne, Victoria, Australia; bDepartment of Epidemiology and Preventive Medicine, Monash University, Melbourne, Victoria, Australia; cSchool of Medicine, University of Adelaide, Adelaide, South Australia, Australia

Background: Serum NT-proBNP level predicts heart failure. The SCREEN-HF study is a community-based cohort study that aims to identify an appropriate threshold NT-proBNP level for stratification of individuals into high and low risk for heart failure.

Aims: To assess whether serum NT-proBNP level can predict heart failure risk in an at-risk population.

Methods: We recruited people with at least one risk factor for heart failure: age ≥60 years with one or more of self-reported myocardial infarction or other ischemic or valvular heart disease, arrhythmia, cerebrovascular disease, renal impairment, or treatment for hypertension or diabetes for ≥2 years. Exclusion criteria were known heart failure or left ventricular dysfunction on previous cardiac imaging. Blood was collected from all participants at baseline for measurement of electrolytes, creatinine and NT-proBNP. Median age of the 3938 participants (2171 men and 1767 women) was 70 years (interquartile range 65–75); 83% were receiving treatment for hypertension, 18% were diabetic, 23% had ischemic heart disease (IHD). 11% had cerebrovascular disease, 10% had atrial fibrillation (AF), 32% had body mass index (BMI) >30 kg/m², 7% had obstructive sleep apnoea (OSA), and 23% had glomerular filtration rate <60 mL/min/1.73 m².

Results: At the time of this interim analysis there were 77 cases of incident heart failure (49 men and 28 women) during a median follow-up of 6 years (incidence rate 3.3 per 1000 person years). Relative to NT-proBNP tertile 1, the odds ratio for incident heart failure was 4.0 (95% confidence interval: 1.1–14.4) for tertile 2 and 21.6 (6.8–69.0) for tertile 3. The C-statistic from receiver operating characteristic analysis was 0.81 (0.77–0.86), with similar values for men and women. NT-proBNP >18 pmol/L (the highest 35%) predicted incident heart failure with 80.5% sensitivity, 66.2% specificity, positive predictive value 4.5% and negative predictive value 99.4%. Although age, diabetes, IHD, AF, BMI and OSA were significant predictors of incident heart failure in a multivariable logistic regression model including NT-proBNP, none improved classification of heart failure risk beyond NT-proBNP alone. Among 3046 participants who had echocardiography, NT-proBNP >18 pmol/L predicted left ventricular ejection fraction (LVEF) <45% with 74% sensitivity and LVEF <40% with 79% sensitivity.
Hypertension was reduced by exposure to aversive novel stressors was abolished by a HFD. Thus there appears to be a mutually beneficial relationship in other beds. Renal denervation did not alter the renal impairment or the hypertension, but RSNA baroreflexes in both groups were shifted downwards so that both maximum and minimum RSNA were reduced. The hypertension to hypoxia spillover was unaltered by denervation, but RSNA baroreflexes in both groups were shifted downwards so that both maximum and minimum RSNA were reduced. The hypertension to airjet was enhanced in both groups after denervation, but the hypertension to hypoxia spillover was 32% lower in CKD rabbits (P<0.01). Plasma creatinine and urea were 44% and 38% higher in CKD than control rabbits (P<0.01). BP and RSNA were 8% and 33% higher (P<0.001), but total noradrenaline spillover was 32% lower in CKD rabbits (P<0.05). Responses to hypoxia were attenuated by denervation in CKD but not in controls. Noradrenaline spillover was unaltered by denervation, but RSNA baroreflexes in both groups were shifted downwards so that both maximum and minimum RSNA were reduced. The hypertension to airjet was enhanced in both groups after denervation, but the hypertension to hypoxia was attenuated in the control animals only. Blood parameters were not altered by renal denervation. Conclusion: Our results show that while RSNA is higher in hypertensive humans taking ACEi, the renal nerves play a minor role in maintaining CKD-induced hypertension since chronic renal injury can stimulate afferent sensory nerve fibres which directly modulate central neuronal circuits and thus sympathetic outflow. We investigated a novel conscious rabbit model of chronic impaired renal function in which quantitative comparisons can be made of sympathetic activity. We determined the role of the renal nerves in the hypertension and altered autonomic reflex function which characterize the 5/6 renal nephrectomy model of CKD.

Aim: To determine whether renal nerves play a role in maintaining CKD-induced hypertension.

Methods: Chronic renal failure was induced by lesioning 5/6th of the glomerular layer in young, healthy rabbits. The renal nerves are likely to play a major role in CKD-induced hypertension since chronic renal injury can stimulate afferent sensory nerve fibres which directly modulate central neuronal circuits and thus sympathetic outflow. We investigated a novel conscious rabbit model of chronic impaired renal function in which quantitative comparisons can be made of sympathetic activity. We determined the role of the renal nerves in the hypertension and altered autonomic reflex function which characterize the 5/6 renal nephrectomy model of CKD.

Conclusion: Although the dispensed price of diuretic-based treatment of hypertension in the elderly is lower, upon considering the potential enhanced likelihood of the development of diabetes in addition to the costs of treating cardiovascular disease, ACEI-based treatment may be a more cost-effective strategy in this population.

EFFECT OF A HIGH FAT DIET AND/OR CHRONIC STRESS ON CAROTIDO悉尼社区中高血钾的共同病史是糖尿病。

血浆利钠肽（PI N）水平有助于分层心脏病风险在老年人群中的分组。 Improved identification of individua- biological and behavioral stressors before and after exposure to chronic stress. The cardiovascular response was estimated by blood pressure (BP) and heart rate (HR). After recovery mice were divided into non-stressed and stressed groups. Group A was restricted to participants diabetes-free at baseline (n=5,642); and Group B was restricted to participants with pre-existing diabetes mellitus (Type I or Type II) at baseline (n=441). One-way and probabilistic sensitivity analyses were performed to assess the uncertainty around utilities and cost data.

Results: For Group A, the ICER was AUD (Australians dollars) 27,888 per QALY gained comparing ACEI-based with diuretic-based treatment. In Group B, ACEI-based treatment was a dominant strategy (both more effective and cost-saving). On sensitivity analysis, the ICERs per QALY gained were always below AUD 50,000 for Group B, whereas for Group A the probability of being below AUD 50,000 was 85%.

Conclusions: Although the dispensed price of diuretic-based treatment of hypertension in the elderly is lower, upon considering the potential enhanced likelihood of the development of diabetes in addition to the costs of treating cardiovascular disease, ACEI-based treatment may be a more cost-effective strategy in this population.

ROLE OF THE RENAL NERVES IN A CONSCIOUS RABBIT MODEL OF CHRONIC KIDNEY DISEASE

Head GA, Burke SL, van Rensch LM, Sata Y, Lambert GW, Denton KM, Schlaich MP

Background: Chronic kidney disease (CKD) contributes substantially to the global burden of cardiovascular disease. Nearly 1 in 3 Australians is at risk of developing CKD which is associated with activation of the sympathetic nervous system and elevated blood pressure (BP). The renal nerves are likely to play a major role in CKD-induced hypertension since chronic renal injury can stimulate afferent sensory nerve fibres which directly modulate central neuronal circuits and thus sympathetic outflow. We investigated a novel conscious rabbit model of chronic impaired renal function in which quantitative comparisons can be made of sympathetic activity. We determined the role of the renal nerves in the hypertension and altered autonomic reflex function which characterize the 5/6 renal nephrectomy model of CKD.

Aim: To determine whether renal nerves play a role in maintaining CKD-induced hypertension.

Methods: Chronic renal failure was induced by lesioning 5/6th of the glomerular layer of the renal cortex in one kidney and, after 2 weeks recovery, removing the contralateral kidney. We examined the role of the renal nerves by denervating the kidneys. Blood parameters, BP and renal sympathetic nerve activity (RSNA) and responses to stress (airjet and hypoxia) were measured using magnetic resonance phase contrast imaging. Total cerebral blood flow was the sum of the mean blood flow in all vessels. Total cerebral vascular resistance (CVR) was calculated as mean arterial pressure/total flow during the MR scan.

Results: Average 24 hour ambulatory day-time systolic and diastolic blood pressures in both treated groups were comparable (139±4/91±4 mmHg vs. 141±3/92±3 mmHg) and were higher than systolic pressure in the NT subjects (117±2 mmHg: P<0.05). The ACEI group had a total cerebral blood flow similar to NT (49±2 vs. 50±2 mL/100 ml/mm), whereas the non ACEI group had a lower cerebral blood flow vs. NT (42±2±1 mL/100 ml/mm; P<0.05). Importantly, CVR was increased in the non ACEI group vs. the NT group (1.8±0.1 vs. 1.4±0.2 mmHg•min/L; P<0.05).

Conclusion: The present data provide preliminary evidence that ACEI might help to prevent a reduction in cerebral blood flow in hypertensive humans, potentially by preventing a rise in CVR.

EFFECT OF A HIGH FAT DIET AND/OR CHRONIC STRESS ON CAROTIDOREactivity in Mice


Baker IDI Heart & Diabetes Institute, Melbourne, Victoria, Australia

Background: Chronic stress exposure to a high fat diet (HFD) causes hypertension and increased activity of the sympathetic nervous system (SNS). Exposure to chronic averse stress also influences the SNS and areas in the CNS similar to those activated by a HFD. Thus there is potential for an adverse interaction between a HFD and chronic stress on cardiovascular regulation.

Aim: To investigate the interaction between a HFD and chronic stress on cardiovascular reactivity in conscious mice.

Methods: Male C57Bl6 mice were fed either a normal fat diet (NFD, n=15) or a HFD (n=11) containing 45% of total energy from fat, for 4 months. Blood pressure (BP) and renal sympathetic nerve activity (RSNA) and responses to stress (airjet) and hypoxia were measured using magnetic resonance phase contrast imaging. Total cerebral blood flow was the sum of the mean blood flow in all vessels. Total cerebral vascular resistance (CVR) was calculated as mean arterial pressure/total flow during the MR scan.

Results: Average 24 hour ambulatory day-time systolic and diastolic blood pressures in both treated groups were comparable (139±4/91±4 mmHg vs. 141±3/92±3 mmHg) and were higher than systolic pressure in the NT subjects (117±2 mmHg: P<0.05). Importantly, CVR was increased in the non ACEI group vs. the NT group (1.8±0.1 vs. 1.4±0.2 mmHg•min/L; P<0.05).

Conclusion: The present data provide preliminary evidence that ACEI might help to prevent a reduction in cerebral blood flow in hypertensive humans, potentially by preventing a rise in CVR.

ANTI-HYPERTENSIVE TREATMENT AND CEREBRAL BLOOD FLOW IN HUMAN HYPERTENSION

Hart EC, Ratcliffe LE, Burchell AE, Nightingale AR, Wise R, Paton JFR

School of Physiology and Pharmacology, University of Bristol, Bristol, UK; 2School of Clinical Sciences, University of Bristol, Bristol, UK;

Cardiology, University Hospitals Bristol NHS Trust and Foundation, Bristol, UK; 3Cardiff University Brain Research Imaging Centre, Cardiff University, UK

Background: The onset of high blood pressure may be due to poor cerebral circulation. Additionally, hypertension is related to the development of dementia. Previous work suggests that angiotensin converting enzyme inhibitors (ACEI) improve cerebral blood flow and prevent pathological remodelling of the cerebral vessels when administered to young, hypertensive animals. Aim: To measure whether cerebral blood flow was higher in hypertensive humans taking ACEI.

Methods: The study involved comparison of hypertensive subjects being treated with ACEI (n=14 [9 being men], age: 58±8 years) and hypertensive subjects taking other anti-hyper-tensive medications (n=12 [7 being men], age 57±6 years), comprising 6 on calcium channel blockers and 6 on angiotensin receptor blockers) and normotensive (NT) participants (n=13, 54±6 years). Blood flow in the left and right internal carotid and vertebral arteries was measured using magnetic resonance phase contrast imaging. Total cerebral blood flow was the sum of the mean blood flow in all vessels. Total cerebral vascular resistance (CVR) was calculated as mean arterial pressure/total flow during the MR scan.

Results: Average 24 hour ambulatory day-time systolic and diastolic blood pressures in both treated groups were comparable (139±4/91±4 mmHg vs. 141±3/92±3 mmHg) and were higher than systolic pressure in the NT subjects (117±2 mmHg: P<0.05). The ACEI group had a total cerebral blood flow similar to NT (49±2 vs. 50±2 mL/100 ml/mm), whereas the non ACEI group had a lower cerebral blood flow vs. NT (42±2±1 mL/100 ml/mm; P<0.05). Importantly, CVR was increased in the non ACEI group vs. the NT group (1.8±0.1 vs. 1.4±0.2 mmHg•min/L; P<0.05).

Conclusion: The present data provide preliminary evidence that ACEI might help to prevent a reduction in cerebral blood flow in hypertensive humans, potentially by preventing a rise in CVR.

THE CONTRIBUTION OF OGREXIN TO THE NEUROGENIC HYPERTENSION IN BPH/2 MICE


Neuropharmacology Laboratory, Baker IDI Heart and Diabetes Institute, Melbourne, Victoria, Australia; 2School of Medical Sciences, University of New South Wales, Sydney, New South Wales, Australia

Conclusions: Serum NT-proBNP level assists stratification of heart failure risk among a community population with risk factors for heart failure. Improved identification of individu-
Background: Schlagner BH/2J mice are a genetic model of hypertension associated with an overactive sympathetic nervous system (SNS). This is associated with a central and peripheral sympathetic overactivity, blood pressure and stress. Importantly, levels of orexin precursor mRNA are greater in the hypothalamus of BH/2J mice compared with normotensive PW/3J control mice, particularly during the dark period of the 24 h light cycle when hypertension is at its greatest in these mice.

Aims: To determine whether enhanced orexergic signaling contributes to hypertension in BH/2J mice.

Methods: BH/2J and PW/3J mice (n=6–7) were pre-implanted with radiofrequency probes to measure mean arterial pressure (MAP). The dual orexin receptor 1 and 2 antagonist, almorexant (Actelion Pharmaceuticals Ltd) was administered via intraperitoneal injection (i.p.) and gavage (p.o.). MAP was recorded for 6 h and compared with baseline values 1 h before treatment. Mid frequency (0.3–0.5 Hz) MAP power and the depressor response to ganglion blockade were both used as indicators of SNS activity in vehicle and Almorexant (i.p., 100 mg/kg)-treated mice (n=3–4).

Results: Administration of almorexant at 100 mg/kg (i.p.) and 300 mg/kg (p.o.) during the dark period of the 24 h light cycle caused sustained hypotensive responses in BH/2J mice (−15.1±1.4 to −10.4±1.1 mm Hg, respectively), which were markedly greater than the effect of vehicle administration (−0.5±0.7 mm Hg; P<0.001). By contrast, the responses to almorexant in PW/3J mice at all doses and routes were comparable with vehicle (P>0.57). During the dark period almorexant attenuated the depressor response to ganglion blockade in both BH/2J and PW/3J mice (P<0.007). Almorexant treatment also reduced the mid frequency MAP power in BH/2J mice (P<0.001), but not PW/3J mice (P=0.65). During the light period, almorexant (100 mg/kg, i.p.) did not reduce MAP from baseline in either strain, but a moderate pressor effect following vehicle injection was reduced following almorexant treatment (13.3±1.7 mm Hg vs 1.6±2.0 mm Hg, respectively; P<0.001) in PW/3J mice only.

Conclusion: These results demonstrate that inactivation of orexin 1 and 2 receptors with almorexant delivered either orally or i.p. can reduce BP and SNS activity in BH/2J mice. These findings suggest that enhanced orexergic signaling contributes to overactivation of the SNS and hypertension in BH/2J mice, particularly during the dark period.

BARORECEPTOR FUNCTION IS PRESERVED FOLLOWING FIELD STIMULATION OF CAROTID BARORECEPTORS IN NORMOTENSIVE AND HYPERTENSIVE RATS

Kouchaki Z1, Buttin M1, Georgakopoulos DP1, Avolio AP1
1Australian School of Advanced Medicine, Macquarie University, Sydney, New South Wales, Australia; 2Olivo Inc., Minneapolis, Minnesota, USA

Background: Field stimulation of the carotid baroreceptors has been used successfully to induce long-term reduction in blood pressure. However, whether baroreceptor stimulation may affect the short-term blood pressure regulation function of the baroreceptors in normotensive and hypertensive conditions is not well established.

Aims: To determine the effect of field stimulation of carotid baroreceptors on blood pressure in normotensive and hypertensive rats.

Methods: Male Wistar Kyoto (WKY, n=7) and spontaneously hypertensive rats (SHR, n=7), in normotensive and hypertensive conditions, were anesthetized (urethane, 1.3 g/kg) and unilaterally vagotomized. Field stimulation was applied to baroreceptors in the proximity of the carotid bifurcation under sterile conditions. PVW was not change significantly with stimulation in both WKY and SHR. Gain during stimulation, 0.6±0.12 bpm/mm Hg; gain during stimulation, 0.44±0.12 bpm/mm Hg; gain during stimulation, 0.6±0.12 bpm/mm Hg; gain during stimulation, 0.6±0.12 bpm/mm Hg. SHR: gain at baseline (no stimulation), 0.7±0.19 bpm/mm Hg; gain during stimulation, 0.6±0.12 bpm/mm Hg; SHR: gain at baseline (no stimulation), 0.36±0.10 bpm/mm Hg; gain during stimulation, 0.44±0.12 bpm/mm Hg. There was a non-significant trend for reduction in gain in SHR compared to WKY in both baseline and stimulation conditions. PVW did not change significantly with stimulation in both WKY and SHR.

Conclusions: Baroreceptor function was preserved during field stimulation of carotid baroreceptors in both WKY and SHR. This provides support for the use of field stimulation of baroreceptors as a means of blood pressure lowering therapy, whereby the acute and transient control of blood pressure in daily life is maintained.

INFLAMMASOME ACTIVITY IS ESSENTIAL FOR DEOXYCORTICOSTEROINE ACETATE/SALT-INDUCED HYPERTENSION IN MICE

Krahmann SM1, Sobei GD1, Kemp-Harper B1, Chan CT2, Diep H2, Dowling J, Finairi A1, Mansell A2, Drummond GR2
1Department of Pharmacology, Monash University, Melbourne, Victoria, Australia; 2Centre of Innate Immunity and Infectious Diseases, Monash Institute of Medical Research, Melbourne, Victoria, Australia

Background: Inflammammasomes are signaling complexes comprised of a NOD-like receptor (ASC) and caspase-1. Inflammammasomes detect host-derived danger signals, causing activation of caspase-1, which in turn cleaves the cytokines pro-interleukin (IL)-1β and pro-IL-18 into their active, pro-inflammatory forms. Inflammammasome activity is associated with chronic renal inflammation, but the role of the inflammasome in the kidney, and assess the impact of inhibition of inflammasome activity on blood pressure (BP) and markers of renal inflammation and fibrosis.

Methods and Results: Male C57BL/6J (wild type) and ASC−/− mice were uninephrectomized, implanted with a DOCA pellet (2.4 mg/day, 21 days, s.c.) and had their drinking water replaced with 1% saline (1K/DOCA/salt). Control mice also had a kidney removed but received a placebo pellet and normal drinking water. 1K/DOCA/salt-treated mice had elevated systolic BP (146±4 mm Hg) compared to control mice (115±2 mm Hg; n=13–16; P<0.05). 1K/DOCA/salt-induced hypertension was associated with increased renal mRNA (fold-change vs. control; n=7–9; P<0.05) of inflammasome subunits NLRP3 (2.3±0.2, ASC (2.5±0.6) and pro-caspase-1 (2.6±0.5), and of the cytokine pro-IL-1β (4.0±0.8). Moreover, protein levels of cleaved (active) caspase-1 and IL-1β were increased by 1.6±0.2- and 2.1±0.3-fold, respectively in kidneys of 1K/DOCA/salt vs. control mice (n=6; P<0.05). ASC−/− mice, which lack an active inflammasome complex, displayed blunted hypotensive responses to 1K/DOCA/salt-treatment (140±3 mm Hg) compared to wild types (155±8 mm Hg; n=8–9; P<0.05). ASC−/− mice were also protected from 1K/DOCA/salt-induced increases in renal levels of mRNAs for inflammatory proteins IL-6, IL-17a, CCL2, ICAM-1 and VCAM-1, and accumulation of collagen.

Conclusion: Renal inflammation, fibrosis and elevated BP in response to 1K/DOCA/salt-treatment are critically dependent on inflammasome activity, highlighting this signaling complex and its cytokine products as potential therapeutic targets to treat hypertension.

INVESTIGATING THE ROLE OF B CELLS IN THE VASCULAR WALL DURING ANGIOTENSIN II-INDUCED HYPERTENSION IN MICE

Lieu M1, Chan CT2, Sobei GD1, Kyaw TS2, Tipping P3, Bobik A1, Toh BH2, Drummond GR2
1Vascular Biology & Immunopharmacology Group, Department of Pharmacology, Monash University, Clayton, Melbourne, Victoria, Australia; 2Vascular Biology & Autoimmunology Laboratory, Baker IDI Heart and Diabetes Institute, Melbourne, Victoria, Australia; 3Centre for Inflammatory Diseases, Department of Medicine, Southern Clinical School, Monash University, Clayton, Melbourne, Victoria, Australia

Background: Recent studies by our Laboratory suggest that B cell-deficient mice display a blunted hypertensive response to angiotensin (Ang) II. However, the mechanism(s) by which B cells promote hypertension remain to be determined.

Aims: (i) To examine whether Ang II-induced hypertension in mice is associated with the accumulation and/or activation of B cells within the vascular wall. (ii) To determine whether these cells represent a source of antibodies, chemokines and/or reactive oxygen species (ROS) during hypertension.

Methods: Wild-type (C57BL/6J) and B cell activating factor-receptor knockout (BAFF-R−/−) mice (deficient in B cells) were treated with Ang II (0.7 mg/kg/i.d., s.c.) or vehicle (0.9% saline) for 14 days. Systolic blood pressure (BP) was monitored via tail cuff and, at day 14, mice were killed, and aortas, serum and spleens were collected.

Results: Ang II treatment for 14 days increased systolic BP in wild-type mice from 118±1 mmHg to 155±3 mmHg (P<0.001; n=25). Although baseline systolic BP in BAFF-R−/− mice was similar to wild-type, the Ang II-dependent pressor effect was blunted (137±4 mmHg; P<0.05; n=13). Taqman real-time PCR revealed that while aortic mRNA levels of pan-B cell markers, CD19 and CD20, were unchanged in Ang II- versus vehicle-treated wild-type mice, B cells were a potential source of these chemokines. A PCR screen suggested that Ang II-induced hypertension in wild-type mice was associated with upregulation of several chemokines in the aorta including CCL2, CCL5, CCL7, CCL8 and CCL12 (1.5- to 6-fold, n=3). Using Taqman real-time PCR, we confirmed these findings (1.5-fold; P<0.05; n=6) and further showed that in BAFF-R−/− mice the Ang II-induced increases in CCL2, CCL5, CCL7, CCL8 and CCL12 were attenuated (n=6), indicating that B cells were a potential source of these chemokines. A Luminex immunoassay demonstrated that serum IgG2a and IgG2b antibodies tended to be elevated (2.0-fold in Ang II- versus vehicle-treated wild-type mice but not BAFF-R−/− mice (P=0.07; n=8–10). Likewise, L-012-enhanced chemiluminescence revealed that spleenic B cells from Ang II-treated wild-type mice produced twice the amount of basal superoxide as those from vehicle-treated mice (P<0.05; n=9–11).

Conclusions: Despite finding no evidence of B cell accumulation in the aorta, B cells from Ang II-treated wild-type mice appear to be more activated and potential sources of antibodies, chemokines and superoxide, which together may contribute to vascular inflammation and dysfunction during hypertension.

MATERNAL OBESITY AND THE DEVELOPMENTAL PROGRAMMING OF HYPERTENSION: THE ROLE OF LEPTIN IN THE CENTRAL NERVOUS SYSTEM

Lim K, Burke SL, Davenport PJ, Head GA
Baker IDI Heart & Diabetes institute, Melbourne, Victoria, Australia
Medical Research Institute, Newcastle, New South Wales, Australia; cGriffith Health Institute, USA) were used for the identification of the following gene polymorphisms: AGT SNPs. These SNPs have been identified in non-Indigenous people as being associated with cardiovascular disease in non-Indigenous people might contribute to the increased risk of cardiovascular and renal disease in Indigenous Australians. To determine the contribution of ACE2 to the regulation of arterial pressure and immune cell infiltration during pregnancy.

Methods: Mean arterial pressure (MAP) was measured via telemetry in 14 week old wild-type (WT) and ACE2 knockout (ACE2-KO) mice receiving vehicle (saline, s.c.; n=9, respectively) or the MasR agonist, AVE-0991 (24 µg/kg/mouse, s.c; n=7, respectively) prior to and during pregnancy. In additional cohorts, renal excretory function was measured via collection of a 24 h urine sample and renal angiotensin receptor (AT1, AT1B, AT2R and MasR) mRNA levels were determined by real-time RT-PCR at baseline and on gestation day (Gd) 18. Circulating immune system activation and immune cell infiltration into the kidneys (baseline and Gd18) and placenta (Gd18) were examined using flow cytometry.

Results: Basal MAP was higher in ACE2-KO versus WT mice (101±1 and 94±1 mm Hg, respectively; P=0.004). Treatment with AVE-0991 lowered basal MAP by 5±1 mm Hg in ACE2-KO mice (P=0.007). In WT mice, MAP decreased during mid-gestation, reaching a nadir at Gd9 (88±1 mm Hg; P=0.004) and returning to near pre-conception levels during late gestation. Whilst the normal gestational decrease in MAP was observed in ACE2-KO mice, MAP increased significantly during late gestation (P<0.0001 vs. WT–vehicle). In ACE2-KO mice treatment with AVE-0991 prevented this late gestation increase in MAP (P<0.0001 vs. WT–vehicle). Basal circulating T regulatory cells were reduced in ACE2-KO mice (P=0.03). Moreover, the reduction in circulating T regulatory cells during pregnancy was greater in ACE2-KO than WT mice (P=0.04). At Gd18, gestational weight gain was lower in ACE2-KO mice compared to WT mice (15±1 vs. 10±1, respectively; P=0.01). At birth, litter size was lower in ACE2-KO mice than WT mice (7±1 vs. 5±1, respectively; P=0.04). AVE-0991 did not affect litter size or birth weight.

Conclusions: MasR stimulation restored the normal arterial pressure pattern during pregnancy in ACE2 deficient mice without an effect on fetal wellbeing. The ACE2/AngII–/–MasR pathway may represent a new therapeutic target for the treatment of pregnancy-induced hypertension.

TREATMENT WITH THE MAS RECEPTOR AGONIST, AVE-0991, RESTORES THE NORMAL REGULATION OF ARTERIAL PRESSURE IN ACE2 DEFICIENT MICE

Mirabito KM, Virah AV, Kruger MA, Tickle C, Widdop RE, Denton KM

1Department of Physiology, Monash University, Melbourne, Victoria, Australia; 2Department of Pharmacology, Monash University, Melbourne, Victoria, Australia; 3Baker ID Heart and Diabetes Institute, Melbourne, Victoria, Australia

Background: Pregnant women and gravid animal models of pregnancy are less sensitive to the pressor effects of angiotensin (Ang) II. Notably, angiotensin converting enzyme (ACE)–2 and Ang (1–7) plasma levels are increased during pregnancy, suggesting that the ACE2/ Ang(1–7)/MasR pathway counters the pressor actions of Ang II during pregnancy.

Aim: To determine the contribution of ACE2 to the regulation of arterial pressure and immune cell infiltration during pregnancy.

Methods: ACE2-KO mice were crossed with WT females to produce offspring of both genotypes. Male offspring were weaned at 3 weeks and housed with female WT or ACE2-KO mice, to create WT crossbreed mice with normal or ACE2-deficient mothers. Blood pressure, heart rate and renal excretory function were measured using radiotelemetry probes. Histological analysis of kidney and placenta was performed. Renin and MasR expression in kidney, placenta, and aorta were measured using real-time RT-PCR.

Results: Treatment with AVE-0991 normalized MAP in ACE2 deficient mice without an effect on fetal wellbeing. The ACE2/AngII–/–MasR pathway may represent a new therapeutic target for the treatment of pregnancy-induced hypertension.

TREATMENT WITH THE MAS RECEPTOR AGONIST, AVE-0991, RESTORES THE NORMAL REGULATION OF ARTERIAL PRESSURE IN ACE2 DEFICIENT MICE

Methods: The study supports our hypothesis of involvement of renal sympathetic nerve activity in the regulation of blood pressure in hypertensive through mechanisms involving miR-181a and renin.
Hypertension Vol 65, No 5 May 2015

Naira P, Velkoska E, Burrell LM, Charchar FJ

*School of Health Sciences, Federation University Australia, Ballarat, Victoria, Australia; 
+Department of Medicine, The University of Melbourne, Austin Health, Melbourne, Victoria, Australia

Introduction: Cardiovascular disease is the major cause of death in patients with kidney disease. Cardiac fibrosis and hypertrophy are common in patients with kidney injury disease, and can be partially attenuated using blockers of the renin-angiotensin system (RAS).

MicroRNAs are a class of small noncoding RNAs that modulate gene expression at the post-transcription level. They play an important role in regulating cell death pathways and cardiac hypertrophy, but is not known whether cardiac microRNAs contribute to cardio-renal cross talk. microRNA-1 and microRNA-133 are associated with cardiac fibrosis and apoptosis after myocardial infarction, whilst microRNA-21/125 is associated with cardiac hypertrophy.

Aims: (i) To investigate, using a rat model, whether acute kidney injury leads to changes in levels of cardiac microRNA-1, -133, -212 and -132 and their mRNA targets. (ii) To test the effect of treatment with the angiotensin converting enzyme inhibitor (ACEI) ramipril on these cardiac microRNAs.

Methods: Heart tissues were collected from rats 10 days after subtotal nephrectomy (STNx), in which one kidney was removed and the other was partially ligated (n=8), from sham animals (n=9) and from STNx rats treated with ramipril (n=9). RNA was extracted from the left ventricle and quantitative real-time PCR was used to measure the microRNAs and their target transcript levels.

Results: In rats with renal injury, there was a significant increase in left ventricular hypertrophy (P=0.05 vs. sham). There was a significant increase in cardiac microRNA-212 (2-fold; P=0.05 vs. Sham) and microRNA-132 (2-fold; P=0.05 vs. Sham). Ramipril treatment in STNx rats attenuated the increase in microRNA-212 and caused a significant increase in microRNA-133 (P=0.001 vs. Sham; P=0.001 vs. STNx+Veh) and microRNA-1 (P<0.01 vs. Sham; P=0.01 vs. STNx+Veh). Renal injury induced left ventricular hypertrophy was attenuated in ramipril treated STNx rats (P=0.001 vs. STNx+Veh). We also found alteration in miR-133a expression of capase 3, fibronectin collagen 1A1 and forkhead box O3, all known for their involvement in the regulation of apoptosis, fibrosis and hypertrophy in cardiac cells, whilst being targets for the above microRNAs.


A NOVEL INSERTIONAL SOMATIC KCNJ5 MUTATION IN AN AUSTRALIAN PATIENT WITH AN ALDOSTERONE-PRODUCING ADENOMA

Stowasser M*, Xu S†, Hardege P†, Murthy M†, Gordon RD‡, O’Shaughnessy KM‡

*Endocrine Hypertension Research Centre, University of Queensland School of Medicine, Greenslopes and Princess Alexandra Hospitals, Brisbane, Queensland, Australia; †Clinical Pharmacology Unit, Department of Medicine, University of Cambridge, Addenbrooke’s Hospital, Cambridge, UK

Background: Primary aldosteronism (PA), in which there is excessive and autonomous production of aldosterone by one or both adrenal glands, accounts for around 5–10% of hypertension. PA may be unilateral (usually due to aldosterone-producing adenoma (APA) and correctable by unilateral adrenalectomy) or bilateral (usually treated medically with agents that antagonize aldosterone action). Recent, somatic mutations in the gene KCNJ5 (encoding a potassium channel) have been detected in about 40% of surgically removed APAs.

Aims: To screen for additional somatic mutations in KCNJ5 in a cohort of APAs removed from 87 Australian patients.

Methods: The full-length coding sequence and flanking regions of KCNJ5 in a cohort of APAs were resequenced. Functional changes caused by a novel mutation were studied in vitro by expressing wild-type (WT) or the mutant KCNJ5 channel in Xenopus oocytes (to examine electrophysiological effects) and by transferring empty GFP vector or the GFP-tagged mutant channel in human adrenocortical carcinoma (H295R) cells (to assess aldosterone release).

Results: KCNJ5 mutations were detected in 37 APAs, and included the previously reported E1450 (n=3), G151R (n=20) and L168R (n=13) mutations plus a novel 12-bp mutation, c.414-425dupGCCCTTCCTGTT (A139_F142dup) that duplicates the AFL sequence just upstream of the selectivity filter. No mutations were found in adjacent cortices. On expression in Xenopus oocytes, the A139_F142dup mutation reduced the resting membrane potential and showed a substantial loss of channel selectivity for potassium (KNa permeability ratio 31 in WT KCNJ5 channels vs. 7 in the A139_F142dup mutant). When transfected into H295R cells, A139_F142dup increased basal aldosterone release 2.3-fold compared to WT. This was not increased further by incubation with angiotensin II. Clinically, the 54-year-old male from whom the mutation-bearing APA was removed had relatively severe PA with resistant hypertension, markedly elevated aldosterone/renin ratio (aldosterone 490 pmol/L and renin 2 mU/L; ratio 296) and an 11 mm left adrenal tumour on CT with lateralization to that side on adrenal venous sampling.

Conclusions: Resequencing of a large Australian cohort of patients with APA further confirmed the major role of KCNJ5 somatic mutations in APA. The novel duplication mutation reported here we report here has similar functional effects to the other mutations affecting the selectivity filter of the KCNJ5 channel with reduced membrane polarization, reduced selectivity to K and increased aldosterone release.

MicroRNAs MEDIATE THE PROTECTIVE EFFECT OF ANGIOTENSIN CONVERTING ENZYME INHIBITION (ACEI) ON THE HEART IN A RAT MODEL OF ACUTE KIDNEY INJURY


National University of Singapore, Singapore; St George’s Hospital Medical School, University of London, London, UK; New York University School of Medicine, New York, USA; National Heart Institute, Singapore; National University of Singapore, Singapore; National University of Singapore, Singapore; National University of Singapore, Singapore; National University of Singapore, Singapore; National University of Singapore, Singapore; National University of Singapore, Singapore; National University of Singapore, Singapore

Background: Acute kidney injury (AKI) is a major cause of death and morbidity in intensive care patients. The full extent of the renal cross-talk with the heart is not yet known. The aim of this study was to investigate the effect of treatment with the angiotensin converting enzyme inhibitor (ACEI) ramipril on heart structure and function in a rat model of AKI.

Methods: C57BL/6J male rats were divided into 3 groups: sham (n=10), AKI (n=10) and ramipril-treated AKI (n=10). AKI was induced by i.v. injection of ischemia-reperfusion injury inducing molecule (IRIM) at 2 mg/kg body weight at 12 h before sacrifice. Ramipril was administered for 14 days at a dose of 1 mg/kg daily. MicroRNA expression was assessed by qPCR. MicroRNA-mRNA target expression was evaluated using a target screening approach.

Results: AKI rats showed a significant decrease in ejection fraction (P<0.05 vs. sham) and a significant increase in microRNA-122 (P<0.05 vs. sham). Ramipril treatment in AKI rats significantly decreased microRNA-122 (P<0.05 vs. sham) and significantly increased microRNA-133a (P<0.05 vs. sham) and microRNA-21 (P<0.05 vs. sham) compared to sham and AKI rats. These changes were accompanied by a significant decrease in mRNA target expression of the genes encoded by these microRNAs.

Conclusions: This study demonstrates that treatment with ACEI can attenuate the deleterious effects of AKI on heart structure and function.

TEN-YEAR EFFECTIVE EFFICACY OF BASELINE BLOOD PRESSURE TREATMENT ON NAIVETY IN THE SECOND AUSTRALIAN NATIONAL BLOOD PRESSURE TREATMENT STUDY.

Nelson MF†, Chowdhury DK‡, Dourt †, Reid CM†, Wing LM*†

*Menzies Research Institute Tasmania, University of Tasmania, Tasmania, Australia; †Monash University, Melbourne, Victoria, Australia; ‡Bond University, Gold Coast, Queensland, Australia; †Flinders University, Adelaide, South Australia, Australia

Background: Guidelines recommend that treatment thresholds for blood pressure (BP) be based on absolute cardiovascular disease (CVD) risk. Clinicians are concerned that leaving untreated BP below the threshold to treat long-term adverse cardiovascular events.

Aims: To examine differences in CVD events at 10 years between those who entered a large-scale randomized controlled BP study (ANBP2) after withdrawing from existing long-term therapy (“on-treatment”) and those who were “treatment-naive” in order to help resolve this concern.

Methods: All participants entering the ANBP2 study who did not have a history of a CVD event at baseline were included in the analysis. Cox-regression hazard models, adjusted for clustering and for potential risk factors, were used to assess the effects of previous BP lowering medication use on different study endpoints within the ANBP2 clinical trial (such as any first cardiovascular events, stroke, myocardial infarction, heart failure, cardiovascular mortality and all-cause mortality). An extended 10-year follow-up analysis for cardiovascular mortality and all-cause mortality was also conducted.

Results: We identified 5,378 participants (aged 65–84 years; 52.5% women) who had no prior CVD events. No difference in fatal CVD events (hazard ratio [HR] 0.96; 95% CI 0.79–1.16; P=0.65) or all-cause mortality (HR 0.96; 95% CI 0.83–1.11; P=0.58) between treatment-naive and on-treatment groups was observed after a median of 10.8 years and therefore no long-term evidence of a legacy effect was observed. We did find lower in-trial HR for fatal CVD events (HR 0.52; 95% CI 0.27–0.81; P=0.007) and all-cause mortality (HR 0.63; 95% CI 0.46–0.86; P=0.004) for the treatment-naive compared to the on-treatment group. This was observed despite the treatment-naive group having a poorer CVD risk profile. By looking for temporal effects, we investigated whether this was an effect of the initial protocol, since individuals on-treatment had gone through a drug withdrawal program prior to randomization, but this did not explain the observed differences.

Conclusions: We found no evidence for long-term adverse CVD risk associated with delayed treatment of elevated BP in an elderly hypertensive cohort. Observed differences between the groups may suggest “healthy survivor” effects could be at play. Legacy effects need to be further explored in randomized placebo controlled trials of middle-aged populations, as this is the population of clinical concern.

Downloaded from http://hyper.ahajournals.org/ by guest on August 30, 2017
BIOMIMETIC VASCULAR DISEASE MICROCHIPS FOR THERAPEUTIC AND BIOPHARMACEUTICAL RESEARCH
Tan A Matsunaga Y
Center for International Research on Integrative Biomedical Systems, Institute of Industrial Science, The University of Tokyo, Tokyo, Japan
Background: Bottom-up vascular tissue engineering is a health-enabling technology pivotal to new ventures in regenerative medicine, pharmaceutical studies and therapeutic research.
Aim: To report on the fabrication of a three-dimensional, tubular co-culture construct that closely simulates the human vascular morphology, both in healthy and inflammatory conditions, to facilitate therapeutic and biopharmaceutical research.
Methods: The co-culture vascular model comprises a neo-intima (constructed using human aortic smooth muscle cells, SMC) covered by a monolayer endothelial lining (i.e., human umbilical vein endothelial cells [EC]). The vascular cells are seeded step-wise along the contour of collagen gel microchannels (120-300 μm in diameter) hosted in a polydimethylsiloxane (PDMS) chamber. Each cell layer is cultivated for 2 days to reach confluence at 37°C/5% CO2. The healthy-state vascular construct is subsequently transformed into a disease model by creating the dynamic microenvironment of inflammatory vascular conditions, mimicking the formation of atherosclerotic lesions. The typical pathophysiological events are initiated via sequential influxes of pro-inflammatory cytokines (human TNF-alpha), low density lipoproteins (LDL) and monocytes/macrophages. Alternatively, LDL-loaded monocytes/macrophages are co-incubated with the SMC/EC to generate intussuscepted foam cells (i.e., monocytes/macrophages rich in LDL particles transmigrated into the sub-endothelial space) instantaneously. Optical and confocal microscopy imaging techniques are used to visualize the three-dimensional, tubular co-culture construct, as well as the formation of foam cells as observed in the early stage of atherosclerosis.
Results: The technique described will be used to assess the vascular effects of various compounds.
Conclusion: The disease-mimicking inflammatory vascular microchips will serve as a powerful high-throughput preclinical tool to probe the localized therapeutic actions and biopharmaceutical performance of various vascular-protective agents and pharmaceutical formulated compounds.

RANDOMIZED CONTROLLED TRIAL ON THE EFFECT OF VITAMIN D SUPPLEMENTATION ON PERIPHERAL AND CENTRAL BLOOD PRESSURE, VISIT-TO-VISIT BLOOD PRESSURE VARIABILITY AND AORTIC STIFFNESS IN OLDER INDIVIDUALS
Venotui P, Sharman JE, Blizzard CL
Menzies Research Institute Tasmania, University of Tasmania, Hobart, Tasmania, Australia
Background: Observational studies report inverse relationships of serum vitamin D with blood pressure (BP) indices and aortic stiffness. This suggests that vitamin D supplementation may have cardiovascular benefits, especially in people with low vitamin D, but there is limited intervention data to support this hypothesis.
Aim: To determine the effect of vitamin D supplementation on BP indices (including BP variability [BPV]) and aortic stiffness in people with vitamin D deficiency.
Methods: In a double-blind, placebo-controlled trial, 239 older individuals (aged 63±7 years; female 50%) with vitamin D deficiency (<50 and >12.5 nmol/L) were randomized to 12-months intervention; vitamin D3, 50,000 IU/month; n=118) or matching placebo (n=121). Brachial and central BP, as well as visit-to-visit BPV and aortic stiffness (carotid-femoral pulse wave velocity) were measured at baseline, 6 and 12 months.
Results: There was a significant increase in serum 25-hydroxy-vitamin D with intervention compared to placebo (45.1 [96% CI 40.2–49.9] vs. 8.5 [96% CI 3.8–15.2] nmol/L, P<0.001). However, intervention failed to produce any clinical or statistically significant changes in brachial systolic BP (–2.79 [95% CI –5.40 to –0.18] vs. –3.25 [–5.89 to –0.61] mmHg; P=0.8), central BP or BPV indices (all P>0.05), or aortic stiffness (–0.22 [95% CI –0.59 to 0.14] vs. 0.08 [–0.32 to 0.43] m/s; P=0.30).
Conclusion: Despite not observing a significant effect of vitamin D supplementation on arterial blood pressure or aortic stiffness, long-term intervention yielded no improvement in older people with vitamin D deficiency. These results do not support use of vitamin D supplementation to improve cardiovascular health in this population.

CARDIAC ACTIN-MYOSIN CROSS-BRIDGE DYSREGULATION OCCURS EARLY IN THE PATHOGENESIS OF TYPE 2 DIABETIC CARDIOMYOPATHY
University of Victoria School of Medicine, Victoria, Canada
Background: Type 2 diabetes mellitus is a global epidemic. Individuals with IR and T2D have a significantly increased risk of developing cardiovascular disease, including diabetic cardiomyopathy. Diastolic dysfunction associated with diabetic cardiomyopathy is often attributed to increased collagen deposition, extracellular matrix accumulation and hypertrophy. Moreover, impaired diastolic function often occurs ahead of structural remodelling early in the pathogenesis of diabetic cardiomyopathy. Currently, the precise molecular mechanisms leading to the development of early contractile dysfunction in diabetic cardiomyopathy remain largely unclear. One possible contributor to early contractile dysfunction could be myofilament dysfunction. Using synchrotron radiation as a source for small angle x-ray scattering (SAXS), it is possible to evaluate real time cardiac actin-myosin cross-bridge (CB) dynamics in the in situ beating heart simultaneously with cardiac function by left pressure-volumetry. The Goto-Kakizaki (GK) rat is a model of progressive T2DM and by 10–12 weeks of age has established IR and mild hyperglycaemia.
Aim: To determine if IR resulted in impaired CB dynamics and cardiac dysfunction in young, insulin resistant GK rats.
Methods: Evaluation of CB dynamics was performed the in situ beating hearts of GK rats (10–12 weeks old, n=6) and age-matched Wistar control rats (n=7) at the beamline 40XU, SPring-8 Synchrotron. Under surgical anaesthesia, rats were thoracotomized and myocardial SAXS patterns were digitally recorded during baseline conditions and dobutamine stimulation.
Results: GK rats displayed cardiac hypertrophy and moderate hyperglycaemia compared to Wistar rats. In the subendocardial and subepicardial layers of the heart, diastolic myosin head proximity to actin filaments was significantly reduced (P<0.05 and P=0.01, respectively) in comparison to Wistar rats, but was normalised during dobutamine infusion. As the calculated spacing between the myosin filaments did not differ between Wistar and GK rats, our data suggests diastolic myosin head extension is depressed in the deeper myocardial layers of the insulin resistant heart.
Conclusions: In the heart of young GK rats, CB dynamics in the subendocardium and subepicardium are slowed by increased separation of myosin heads and may be an early event that drives diastolic dysfunction in the diabetic heart.

ANGIOTENSIN CONVERTING ENZYME 2 DEFICIENCY PROMOTES AORTIC ANEURYSM FORMATION AND RUPTURE IN APOLIPOPROTEIN E-DEFICIENT MICE
FH University of Australia, Ballarat, Victoria, Australia; aBaker ID Heart & Diabetes Institute, Melbourne, Victoria, Australia; bJames Cook University, Townsville, Queensland, Australia
Background: Angiotensin (Ang) II has been implicated in aneurysm development and rupture. Angiotensin converting enzyme 2 (Ace2) is the major enzyme that metabolizes Ang II within vascular tissue. The expression of Ace2 is down-regulated at least 7-fold in the aorta of patients with abdominal aortic aneurysms when compared to healthy tissue from donors (P=0.025). We hypothesise that this down-regulation may contribute to the development and progression of aortic aneurysms.
Aim: To determine whether down-regulation of Ace2 mRNA in aorta contributes to development and progression of aortic aneurysms.
Methods: To explore this hypothesis, apolipoprotein (Apo) E gene knockout (KO) mice and ApoE/Ace2 KO mice were treated with Ang II (1 μg/kg/min, s.c.) or vehicle.
Results: At baseline, ApoE/Ace2 KO mice had larger aortic arch and suprarenal aortic diameters. The mRNA levels encoding proteins associated with inflammation and aneurysm formation were increased in the aorta of ApoE/Ace2 KO mice. The mRNA included those encoding ICAM-1, osteopontin, and matrix metalloproteinase-2 and -9. At the same time, the activity of the matrix crosslinking enzyme lysyl oxidase was decreased. When Ang II was infused into ApoE/Ace2 KO mice the aortic dilatation was substantially enhanced compared to control animals. In addition, within 7 days of commencing the infusion, 10 of 12 (83.3%) ApoE/Ace2 KO mice died due to aortic rupture compared to only 2 of 13 control mice (15%; P=0.0004). Fatal rupture was observed as early as day 3 after commencement of Ang II infusion.
Conclusion: Reduced Ace2 expression, as observed in human aneurysms, promotes vascular inflammation, aortic dilatation and rupture in a mouse model of aortic aneurysms. These data support the potential utility of targeting ACE2 in a condition that currently has no medical treatment.

DOES CONTRALATERAL SUPPRESSION AT ADRENA VEIN SAMPLING PREDICT OUTCOME FOLLOWING UNILATERAL ADRENALECTOMY FOR PRIMARY ALDOSTERONISM? A RETROSPECTIVE STUDY
Welley MJ, Ahmed AH, Gordon RD, Stowasser M
University of Queensland School of Medicine, Brisbane, Queensland, Australia
Background: In primary aldosteronism (PA), adrenal vein sampling (AVS) is the most reliable method of distinguishing unilateral from bilateral disease. In AVS aldosterone/cortisol ratios (AVF) are used to correct aldosterone concentration for dilution from non-adrenal blood. Comparisons are then made between left, right and peripheral AVF ratios. Criteria for interpretation vary widely and there is no clear evidence of which method is most accurate. Most units use the lateralization index (LI): AVF dominant: AVF non-dominant with a cut-off value varying from 2:2–4 for unstimulated AVS to indicate unilateral disease. We have for many years used the criteria of ‘contralateral suppression’ (CS), defined as: AVF (adrenal)

Downloaded from http://hyper.ahajournals.org/ by guest on August 30, 2017
≤ AF (peripheral) on the unaffected side, combined with a ratio of ≥ 2 times peripheral on the affected side. Patients with one side clearly dominant but without CS are, however, sometimes offered surgery, depending on their particular characteristics and wishes. The importance of contralateral suppression in AVS interpretation is unclear. **Aim:** To perform a retrospective study to determine if CS in unilateral PA was associated with blood pressure (BP) and biochemical outcomes. **Methods:** All patients who underwent unilateral adrenalectomy for PA at the Princess Alexandra Hospital, Brisbane between 2000 and 2014 were included for review if AVS was successful (based on gradients between adrenal and peripheral cortical of ≥2 bilaterally), if the LI was ≥2 and if they had ≥6 months of post-operative follow-up. Cases were reviewed for BP and biochemical outcomes with respect to the presence and degree of CS. **Results:** 80 patients were available for review, and 66 had CS. Baseline characteristics were similar. At post-operative follow up, those with CS had lower systolic BP (SBP: 129 mmHg vs. 143 mmHg; P=0.001), a greater proportion with cure or improvement of hypertension (95% vs. 64%; P=0.0034), a greater proportion with biochemical cure of PA on fluorod ricortisol suppression testing (43/49 [88%] vs. 4/9 [40%]; P=0.002) and were on a lower median number of antihypertensive medications (0 vs. 1.5; P=0.002). In a multi variate linear model, the degree of CS and pre-operative SBP were both significantly correlated with post-operative SBP, but LI, gender and age were not. **Conclusions:** In this retrospective study of the contribution of CS to the interpretation of AVS, the presence of CS correlated with good BP and biochemical outcomes from surgery. This suggests that CS should be a factor in deciding whether to offer surgery for treatment of PA.

A PILOT INVESTIGATION OF CEREBROVASCULAR RESPONSIVENESS TO A NEUROPSYCHOLOGICAL TEST BATTERY IN ADULTS WITH TYPE 2 DIABETES MELLITUS

Wong RHX, Jansen L, Nealon R, Garg ML, Howe PRC

University of Newcastle, Newcastle, New South Wales, Australia

**Background:** Our research examines the dependence of cognitive performance on cerebrovascular perfusion. Progressive arterial disease in type 2 diabetes (T2D) may predispose individuals to greater risk of premature cognitive decline.

**Aim:** To conduct a pilot cross-sectional study in adults with T2D and age- and gender-matched controls without T2D to see whether limitations in the ability of cerebral vessels to supply blood in response to psychological stimuli predict poorer cognitive performance.

**Methods:** Cognitive tests and transcranial Doppler (TCD) ultrasound assessments of cerebral blood flow were conducted in 31 adults with non-insulin dependent T2D and 13 controls after taking 10 mg of metformin (MBFV) and 10 μg of acetylcholine (ACh) (to measure of arterial stiffness) were averaged over 30 s in both left and right middle cerebral arteries (MCA). We subsequently tested their cerebrovascular responsiveness (CVR) to cognitive stimuli by recording changes in MBFV from pre-test levels during a neuropsychological test battery that assessed the cognitive domains of attentional and executive function, working memory and processing speed.

**Results:** Preliminary analysis showed elevated PI in T2D, which was correlated with age (r=0.303; P=0.048 for left MCA; r=0.324; P=0.034 for right MCA). There were, however, no consistent correlations between markers of T2D (duration, glucose, insulin sensitivity and HbA1c) and basal MBFV, PI or cognitive performance. Reproducibility measures were excellent, e.g., ICC for PI were 0.967 (left) and 0.955 (right). Despite similar MMSE scores (mean score of 28.6±1.4 for both groups), adults with T2D tended to perform poorer in all cognitive tests, particularly in the N-back task (working memory). Importantly, CVR to the N-back task was lower in T2D (P=0.057 (left); P=0.011 (right), independent t-test). This deficit did not, however, correlate with task performance.

**Conclusions:** With the limited data available, we were able to show for the first time that a cognitive deficit in T2D is associated with an impaired ability to supply blood to the anterior brain region in response to the specific cognitive stimulus used. This preliminary result suggests that cerebrovascular disease may contribute to deficits in working memory, executive function or processing speed in those with T2D. The finding has significant clinical implications for self-care health behaviours. We are evaluating more participants to gain a clearer understanding of these relationships.

EFFECTS OF BLOOD PRESSURE LOWERING ON CARDIOVASCULAR RISK ACCORDING TO BASELINE BODY MASS INDEX: A META-ANALYSIS OF RANDOMIZED TRIALS

Ying AJ, Arima H, Neal BC

The George Institute for Global Health, University of Sydney, Sydney, Australia

**Background:** Recent reports have suggested that the benefits of blood pressure lowering in obese people compared to people of normal weight may depend upon the choice of drug.

**Aim:** To confirm or refute these findings from individual studies by conducting a meta-analysis based upon individual patient data using multiple trials included in the Blood Pressure Lowering Treatment Trials Collaboration.

**Methods:** We compared the effects of blood pressure lowering regimens based upon different drug classes for the primary outcome of total major cardiovascular events. Meta-analysis and meta-regressions were used to seek evidence for interactions between treatment and body mass index (BMI) when fitted as either a categorical (<25, 25–30, ≥30 kg/m²) or a continuous variable.

**Results:** Analyses were performed on 135,715 individuals drawn from 22 trials who experienced a total of 14,353 events. For none of the six primary end-points made was there evidence that protection varied by drug class across the three BMI groups studied (all P for trend >0.35). When analysed as a continuous variable, angiotensin converting enzyme inhibitors gave slightly greater protection for each 5 kg/m² elevation in BMI than did calcium antagonists (hazard ratio 0.83; 95% CI 0.89–0.96; P=0.004) or diuretics (0.93; 95% CI 0.89–0.98; P=0.002). The meta-regressions identified no relationship between BMI category and the risk reduction achieved for a given fall in systolic blood pressure. In contrast to a major prior report, we found no relationship between BMI and the efficacy of calcium antagonists compared to diuretics.

**Conclusion:** These analyses provide little evidence that the selection of a particular class of blood pressure lowering drug will substantially alter outcomes for individuals who are obese compared to those who are lean. (Publication: Lancet http://www.thelancet.com/journals/lancet/article/PIIS0140-6736(14)61171–5)

TH2-PROMOTING CYTOKINE TREATMENT LIMITS BRAIN INJURY AFTER CEREBRAL ISCHEMIA IN Th1-DOMINANT MICE

Zhang S, Kim H, Vitha A, Dummond G, Soby C

Department of Pharmacology, Monash University, Melbourne, Victoria, Australia

**Background:** Stroke is the second leading cause of death and leading cause of permanent disability worldwide. Current treatment with recombinant tissue plasminogen activator can only be administered within 4.5 h of stroke onset, benefiting less than 10% of all stroke patients. Following ischemic stroke, inflammation occurs in the brain and is a major contributor to secondary injury and tissue inflation. Previous evidence indicates that Th1 helper-type cytokine immunity is associated with a worse outcome in Th1-dominant versus Th2-dominant mouse strains after stroke. It is, however, unknown whether brain injury and functional deficits can be limited by acute therapy to promote Th2-type immunity.

**Aims:** To test if Th2-promoting cytokines are able to switch the immune response in Th1-dominant C57BL/6 mice to a Th2-dominant phenotype, leading to reduced brain infarct, less inflammation and improved functional outcome after stroke.

**Methods:** Male mice were treated with vehicle, IL-4 or IL-33 (1% bovine serum albumin, 5 μg or 2 μg, respectively, i.p.) 24 h before and 1 h after cerebral ischemia. Mice were anaesthetized with ketamine (80 mg/kg) and xylazine (10 mg/kg) i.p. prior to middle cerebral artery occlusion (1 h). Neurological and hanging wire assessments were performed 24 h after stroke. Brains were removed and frozen brain sections (30 μm) were stained with thionin for infarct analysis. Brain inflating leukocytes were stained and quantified using flow cytometry. Antibiotics (ampicillin and gentamycin, 300 mg/kg and 12 mg/kg, respectively, s.c.) were administered to 6 mice n = 6 in combination with IL-33.

**Results:** Brain infarction was reduced by ~35% by IL-33 or IL-4 treatment, as compared to vehicle (26.1±3.1 mm³, 29.1±3.7 mm³ and 43.8±4.6 mm³, respectively; n=13–16; each P<0.05). Mortality and neurological deficit were, however, exacerbated by IL-33 and IL-4 (n > 18; both P<0.01). Flow cytometric analysis indicated that IL-33 reduced Ly6C+ monocyte numbers in the ischemic brain, as compared to vehicle (227±17 versus 279±23 cells, respectively; n=19–20; P=0.05). Ongoing studies suggest that combined IL-33 and antibiotic therapy improves functional recovery compared with IL-33 alone.

**Conclusions:** The present data indicate that acute administration of Th2-promoting cytokine limits brain injury but exacerbates functional deficit after stroke, possibly due to increased bacterial infections. Post-stroke cytokine therapy may be feasible, used together with antibiotics.

HBPCCR Poster Presentations

PULSE WAVEFORM ANALYSIS IN PATIENTS WITH ATRIAL FIBRILLATION: REPRODUCIBILITY STUDY WITH THE SPHYGMOCOR SYSTEM

Adri N, McGrath J, O Rouke MF

A* Australian School of Advanced Medicine, Macquarie University, North Ryde, Sydney, New South Wales, Australia; bFaculty of Medicine, University of New South Wales, Kensington, Sydney, New South Wales, Australia; cSt Vincent’s Clinic, Sydney, New South Wales, Australia

**Background:** To date, it has been considered suitable to use pulse wave analysis system such as SphygmoCor only in patients with steady sinus rhythm, where patients with atrial fibrillation (AF) are excluded.

**Aim:** To test the applicability of the SphygmoCor and its ensemble-averaging process in these AF patients for providing an aortic waveform and its associated indices.

**Methods:** Patients with AF (11 males, age 83±5 years) were studied in a cardiovascular outpatient setting. Brachial pressures were taken with Korotkov sound method, averaged between two measurements. These values were used to calibrate the ensemble-averaged raw waves measured at the wrist with applanation tonometry. Recordings of the radial pressure were taken over a ten second period on 4–6 occasions. We sought to compare differences in the indices of the aortic pressure waves which were clinically useful for pressure, pressure from wave foot to first systolic shoulder (PI), systolic (ASP), augmented (AP), end-systolic pressure (ESP) and pulse pressure (PP); for time intervals, cycle length (CL), heart rate (HR), ejection duration (ED), time to P1 (T1) and time to ASP (T2);
for non-dimensional indices, augmentation index (AIx), Ax corrected for averaged heart period (AxH), and amplification of pulse height (pulse pressure) between ascending and brachial (PPA).

Results: For all parameters, average values were calculated together with standard deviation (SD) and coefficient of variation (CV = SD/mean–mean) as follows, mean (SD, CV): P1 (34.9, 0.5, 0.01 mmHg), ASP (118.2, 1.0, 0.01 mmHg), AP (123.1, 1.0, 0.09 mmHg), ESP (104.7, 1.2, 0.01 mmHg), APP (47.3, 1.0, 0.02 mmHg), CL (383.5, 35.9, 0.04 msec), ED (268.7, 9.3, 0.03 ms), T1 (981.2, 2.02 ms), T2 (2027.4, 4.0, 0.02 ms), Ax (24.6, 1.7, 0.08 %), AxH/PPA (24.0, 2.1, 0.09 %), PPA (1.34, 0.03, 0.02). The CV were surprisingly small, averaging 0.01 to 0.09 for pressure indices, 0.02 to 0.04 for timing indices, and 0.02 to 0.09 for non-dimensional indices. Such variability was most apparent when the pressure waves were recorded sequentially, with surprisingly similar timing for systole, to peak ejection, and to the peak of pressure.

Conclusion: Results of this study provide a good reason for using pulse wave analysis systems in AF patients. With a single recording, waveforms show far greater similarity than one would expect. However, when multiple waveforms are averaged, the parameters are consistent and reproducible. Therefore the use of pulse wave analysis in AF patients is warranted, at least in stable AF with well-controlled HR.

RELIABILITY OF PULSE WAVE FORM ANALYSIS TO MONITOR NITRATE TOLERANCE

Adji A1, Jiang XP1, Ji W2, Li YS3, Rouze MP4, Dong H5, Peng M6, Liu LS6

1Australian School of Advanced Medicine, Macquarie University, North Ryde, Sydney, New South Wales, Australia; 2Hypertension Center, Department of Cardiology, Fuwai Hospital, Beijing, China; 3PeKing Union Medical College, Chinese Academy of Medical Sciences, Fuwai Hospital, Beijing, China; 4Key Laboratory of Clinical Trial Research in Cardiovascular Drug, Fuwai Hospital, Beijing, China; 5St Vincent’s Clinic, Sydney, New South Wales, Australia

Background: Bosiboredo mononitrates (ISMN) have little or no effect on brachial cuff pressures, but substantial effects on aortic pressures and wave contour. Such changes are attributable to dilation of muscular conduit arteries, and reduction of wave reflection from peripheral arteries. This effect explains therapeutic effects of nitrates, but the effect has never been used to quantify nitrate tolerance.

Aim: To quantify tolerance to ISMN in healthy volunteers through measurement of aortic pressure waveform indices after repeated oral administration of ISMN.

Methods: Eighteen healthy male volunteers (mean age 23±2.5 years) were admitted to Fuwai Hospital and studied over three 24-hour periods—baseline (BL, day zero), day 1—after oral administration of 60 mg ISMN slow release tablet, and at day 6—after oral administration of the same dose at the same time on days 1–6. The radial artery waveform measured by applanation tonometry and calibrated to brachial cuff pressures. Radial pressure was converted to an aortic pressure waveform, and both were ensemble-averaged. Blood levels of ISMN were measured over the 24-hour period on days 1 and 6. Central pressure indices taken over 12 occasions over 24 hours were used to show initial effect of ISMN (at BL), and reduction in nitrate therapeutic effect (tolerance) by comparing indices at day 1 and day 6. Effects of ISMN were studied through changes in aortic pressure between BL and day 1, and tolerance from reduction of these changes between day 1 and day 6. Indices were averaged between 0 to 6 hours after oral administration of ISMN.

Results: ISMN blood levels were similar over the 24-hour period on days 1 and 6. Central pressure indices taken over 12 occasions over 24 hours were used to show initial effect of ISMN (at BL), and reduction in nitrate therapeutic effect (tolerance) by comparing indices at day 1 and day 6. Effects of ISMN were studied through changes in aortic pressure between BL and day 1, and tolerance from reduction of these changes between day 1 and day 6. Indices were averaged between 0 to 6 hours after oral administration of ISMN.

Conclusion: Change in contour and height of the aortic pressure waveform can be used to describe initial effect of ISMN, and to quantify the effects of tolerance after 6 days of administration. Nitrate effects, however, remained substantial after 6 days administration.

SHOULD ALDOSTERONE SUPPRESSION TESTS BE CONDUCTED DURING A PARTICULAR PHASE OF THE MENSTRUAL CYCLE, AND, IF SO, WHICH PHASE?

Ahmed AH1, Gordon RD2, Ward GM2, Kogosov CK2, Stowasser MS2

1The University of Queensland School of Medicine, Brisbane, Queensland, Australia; 2Sullivan & Nicolaides Pathology, Brisbane, Queensland, Australia; 3Greenslopes Hospital, Brisbane, Queensland, Australia

Background: Since levels of renin and aldosterone vary during the menstrual cycle, and are critical criteria for interpretation of aldosterone suppression tests to confirm or exclude primary aldosteronism, it is likely that the outcome will vary depending on the phase of the menstrual cycle. With appropriate information, it will be possible to recommend the best phase of the menstrual cycle for primary aldosteronism in menstruating women.

Aim: To obtain information on the effect of timing within the menstrual cycle on the levels of renin, aldosterone, progesterone, estradiol, cortisol, LH and FSH during fludrocortisone suppression testing (FST).

Methods: In 22 women undergoing FST who experienced regular menstrual cycles, mid-morning upright levels of renin (measured as both plasma renin activity and direct renin concentration), aldosterone (measured by mass spectrometry) and cortisol, progesterone, estradiol, LH and FSH (measured by immunoassay) at the conclusion of the 4 day test were compared, relative to the phase of the cycle. Aldosterone levels in both luteal and follicular groups were compared with those in age-matched males.

Results: Median (range) levels of progesterone (follicular 1 [6–6] vs. luteal 26 [11–42] mmol/l [P<0.0001]) and aldosterone (600 [222–1600] vs. 254 [19–437] mmol/l [P=0.009]) were both higher in nine women (after one of 10 was excluded with anovulatory cycle) studied during the luteal phase of the cycle than in 12 studied during the follicular phase. All women studied during the luteal phase had a positive FST (day 4 midmorning upright aldosterone >165 mmol/l) and all three with negative FST were studied during the follicular phase. There were no significant differences in other parameters measured except FSH, which was higher (P=0.02) during the follicular phase. Aldosterone was significantly higher in women studied in the luteal (but not follicular) phase compared to men (278 [59–386]; P<0.01).

Conclusion: The menstrual cycle may affect the outcome of fludrocortisone and other suppression tests used to diagnose primary aldosteronism. Larger patient numbers and preferably restudy of the same patient in both phases by seated saline suppression testing are needed to clarify this question, and to determine the optimum time in the cycle for testing for primary aldosteronism.

EFFECT OF CYCLIC STRETCH ON ENDOTHELIAL NITRIC OXIDE SYNTHASE AND ASSOCIATED CELL SURVIVAL PATHWAYS IN VASCULAR ENDOTHELIAL CELLS

Avanthanam BRL, Gangoda SYS, Gupta V, Butlin M, Arollo AP

Australin School of Advanced Medicine, Macquarie University, Sydney, New South Wales, Australia

Objectives: Arterial endothelial cells are continuously subjected to mechanical forces through cyclic stretch (strain) due to the periodic change in vessel diameter with pulsatile blood flow. Under physiological conditions, this blood flow promotes activation of endothelial nitric oxide synthase (eNOS) and subsequent nitric oxide (NO) release, a protective mechanism in maintaining vascular homeostasis.

Aim: To determine whether exposure of primary human umbilical vein endothelial cells (HUVECs) to cyclic stretch alters the expression and activity of eNOS and associated survival signaling pathways.

Methods: HUVECs (P7) were grown in fibronectin coated silicone chambers at 37°C with 5% CO2 for approximately 24 hours then subjected to uniaxial cyclic stretch of 1 Hz over 18 hours with stretch magnitude of either 0, 5, 10, 15 or 20% using the SheiniPa mechanical stretch system (Menicon Life Sciences, Japan). Percent cell viability was measured using the Countess automated cell counter. eNOS mRNA and eNOS were measured by quantitative RT-PCR and western blotting assays, respectively. The levels and the activity of Akt and GSK 3β proteins were evaluated using western blotting. GAPDH expression was used as an internal control for comparison.

Results: In response to the cyclic stretch of varying magnitudes, an increase in the eNOS mRNA level at both 5% and 15% CO2 was observed compared to no (0%) cell stretch (P<0.05; n=3). A further increase in eNOS mRNA was observed when the cells were exposed to 20% cyclic stretch (P<0.05; n=3). In accordance with the changes in eNOS gene transcription in cells, a qualitative increase in the eNOS protein was observed at 20% stretch levels. Similarly, Akt level was found to be slightly increased following 10% and 20% stretch. Interestingly, phosphorylation and level of GSK3β, which is downstream of Akt, was enhanced in cells exposed to higher and duration of stretching conditions. No significant difference in cell viability was observed when cells were exposed to either 5% or 20% stretch.

Conclusions: Increased eNOS may implicate an enhanced production of the second messenger NO which regulates the vasodilatation in physiological systems. Our study of HUVECs indicate that cyclic stretch is associated with enhanced eNOS expression and upregulation of associated cellular survival signaling pathways. Further studies in animal models will help to correlate these biochemical changes in the context of increased pulse pressures in hypertension.

USE OF RADIAL PRESSURE PULSE FOR ASSESSMENT OF RELATIONSHIP BETWEEN SUBENDOCARDIAL VIABILITY RATIO AND PLASMA B-TYPE NATRIURETIC PEPTIDE IN HEART FAILURE

Arollo AP1, He D2, Xu L1, Geng N3, Butlin M4

1Australian School of Advanced Medicine, Macquarie University, Sydney, New South Wales, Australia; 2Northeastern University, Shenyang, China; 3Key Laboratory of Medical Image Computing, Ministry of Education, Shenyang, China

Background: The subendocardial viability ratio (SEVR) when computed from the aortic pressure is related to degree of subendocardial myocardial perfusion. In heart failure (HF) cardiac function has increased dependency on myocardial oxygen consumption. Oxygen demand can be estimated from the arterial pressure wave by the systolic tension time index (STI) and oxygen supply from the diastolic time index (OTI). SEVR is computed as the ratio OTI/STI. The severity of HF is also related to plasma levels of B-type natriuretic peptide (BNP). The non-invasive measurement of central aortic pressure from the peripheral (radial/brachial) pressure pulse is based on a generally stable relationship between central and peripheral waveforms.

Downloaded from http://hyper.ahajournals.org/ by guest on August 30, 2017
Aim: To assess the relationship between plasma BNP and SEVR when determined from the ABPM data. We hypothesised that the gold standard for assessing blood pressure (BP) in the general population. The prognostic significance of ABPM and the effect of night time BP medications on nocturnal dipping and morning blood pressure surge (MBPS) have been documented in the general population, but less well-defined in chronic kidney disease (CKD) patients.

Methods: CKD patients (n=105) revealed no significant reduction in MBPS with use of night time medications. In contrast to published studies, the use of night time medications did not influence BP medications (no night time medication vs. night time medication, 19.8±1.5 vs. 20.6±2.0%).

Results: A dipping profile was identified in 49% of CKD patients. There was a significant reduction in nocturnal BP dipping (dipping index (dIP) >0.9) between SEVR from the radial pulse and SEVR from the corresponding derived central aortic pressure. Conclusions: SEVR computed from the radial pulse has an inverse relationship with plasma BNP in HF. Further studies will aim to assess whether the addition of SEVR as a screening parameter will improve the HF discriminating power of BNP measurement.

AMBULATORY BLOOD PRESSURE MONITORING PARAMETERS AND THE EFFECT OF NIGHT TIME BLOOD PRESSURE MEDICATIONS ON NOCTURNAL DIPPING AND MORNING BLOOD PRESSURE SURGE IN PATIENTS WITH CHRONIC KIDNEY DISEASE

Bency B, Ho FM®, Williamson PM®, Praphabarsh S®, Beddoo J, Kelly J, Ong S

Background: Ambulatory blood pressure monitoring (ABPM) is the gold standard for assessing blood pressure (BP) in the general population. The prognostic significance of ABPM and the effect of night time BP medications on nocturnal dipping and morning blood pressure surge (MBPS) have been documented in the general population, but less well-defined in chronic kidney disease (CKD) patients.

Aim: To outline ABPM characteristics in different CKD stages and the effects of night time medications on BP control, nocturnal dipping and MBPS.

Methods: Method: CKD (n=157) patients underwent 24 hour ABPM monitoring (SpaceLabs monitors). 24-hour awake and asleep BP averages and percentage nocturnal dipping were calculated from the ABPM data. A BP fall of ≤10% during sleep defined subjects as non-dippers. MBPS was calculated as the difference between morning BP (average of 4 consecutive readings over 2 hours immediately after awakening) and nadir during sleep (average of at least 2 readings around the lowest BP reading during sleep). The timing of antihypertensive medication use was recorded. The stages of CKD were grouped as mild, moderate and advanced based on eGFR <60 (with or without proteinuria); 30–59, <30 mL/min/1.73 m² respectively. Use of night time medication is defined as taking at least one of the antihypertensive medications after 5 pm.

Results: The average readings for 24-hour awake and asleep BP were 132.6±1.9/76.5±1.4, 136.9±1.7/76.6±1.4, 131.7±2.5/86.8±1.5 vs. 131.3±1.7/60.2±1.0, 134.7±1.6/72.6±1.0, 123.6±2.0/64.5±1.2 vs. 135.9±2.1/72.1±1.1, 138.5±2.0/73.5±1.7, 127.9±3.6/65.2±2.0 mmHg in mild (stage 1 & 2; n=30), moderate (stage 3a and 3b, n=79) and severe CKD (stage 4 and 5, n=58) groups, respectively. A dipping profile was identified in 49% of CKD patients. There was a higher prevalence of non-dipping status in advanced stages of CKD (24%, 56%, and 64%, respectively) in mild, moderate and severe CKD (P<0.05). Administration of antihypertensive medications at bedtime did not significantly influence the dipping pattern of patients across different stages of CKD (no night time medication vs. night time medication, 8.6±1.0 vs. 9.0±1.0%). A subgroup analysis of CKD patients (n=105) revealed no significant reduction in MBPS with use of night time BP medications (no night time medication vs. night time medication, 19.8±1.5 vs. 20.6±2.0%).

Conclusion: Higher prevalence of non-dipping status was observed with advancing stages of CKD. In contrast to published studies, the use of night time medications did not influence dipping status or magnitude of MBPS in CKD.

CONTRASTING EFFECTS OF PRENATAL LIFE STRESS ON BLOOD PRESSURE AND BMI IN YOUNG ADULTS

Bhat S, Belin L, Burrows S, Mori T, Robinson M

University of Western Australia, Perth, Western Australia, Australia

Background: Various environmental stressors in pregnancy have been reported to affect high blood pressure (BP) in adult offspring. However, few studies have examined the effect of prenatal maternal psychological stress on offspring BP and BMI in early adulthood.

Aim: To test the hypotheses that prenatal life stress will be associated with higher BP and higher BMI in a young adult population.

Methods: In 1916 participants regression analyses were used to examine the association between the count of maternal life stress events experienced during pregnancy and offspring BP and body mass index (BMI) at age 20.

Results: Prenatal life stress was positively associated with offspring BMI but inversely associated with systolic BP (P<0.001). After adjustment for confounders such as additional prenatal life stress event reduced offspring SBP by 0.66 mmHg (P=0.013) in those with an average BMI and lowered the odds of systolic (pre)hypertension by 17% (OR=0.83; P=0.008). The inverse relationship between prenatal life stress and adult SBP was stronger in offspring with higher BMI. On the other hand, each unit increase in prenatal life stress score predicted a BMI increase of 0.37 kg/m² (P<0.002).

Conclusions: This study has shown that maternal stress in pregnancy is significantly associated with BMI, but contrary to our hypothesis predicted lower resting systolic BP and lower odds of systolic (pre)hypertension in young adult offspring. The effect of prenatal life stress on BP was accentuated by a higher BMI. Fetal programming events as a result of prenatal stress may underpin these relationships.

VALIDATION OF AMBULATORY CUFF-BASED DEVICES FOR CENTRAL AORTIC BLOOD PRESSURE AND ARTERIAL STIFFNESS MEASUREMENT

Butlin MP, Ni Gaithani, Gaseem A, Awuloo AP

Australian School of Advanced Medicine, Macquarie University, Sydney, New South Wales, Australia; aDepartment of Chemistry and Biomedical Science, Macquarie University, Sydney, New South Wales, Australia

Background: As the utility of non-invasive measurement of central aortic blood pressure parameters grows, the logical next step is for out-of-clinic measurement of aortic blood pressure parameters. Recently, ambulatory blood pressure monitors (ABPMs) have come on to the market with this ability.

Aim: To investigate the accuracy of two ABPM devices that estimate central aortic blood pressure (BP Lab® and AtCor/SunTech Oscar 2) by comparing them in clinic to existing validated devices, the Sphygmocor Xcel (cuff-based) and tonometer-based devices.

Methods: 45 subjects were recruited for the study, with 1 subject being excluded due to technical issues. The remaining subjects were 46±17 years old (30 male) with brachial systolic pressure ranging from 104 to 153 mmHg, diastolic pressures from 63–106 mmHg, heart rate from 48–94 bpm. Measurements were taken in triplicate with each device and averaged for repeated-measures statistical comparison of calculated central aortic systolic, diastolic, and augmentation index (Alx) between devices. Devices were also compared by Bland-Altman representation. Calculated aortic systolic pressure was 4.1±3.3 mmHg higher in the Oscar 2 device than the tonometer-based device (tonometer-based 112±2, Xcel 113±2, Oscar 2 116±2 mmHg, P=0.20). Aortic diastolic pressures were consistent between devices (tonometer-based 79±1, Xcel 80±1, Oscar 2 79±1 mmHg, P=0.067). Aortic Alx was greater in the Xcel, and greater still in the Oscar 2 device compared to the tonometer-based device (tonometer-based 13±2%, Xcel 18±2%, Oscar 2 23±2%, P<0.001). There was little bias seen in the Oscar 2 device compared to the tonometer-based device for either aortic systolic (slope=0.92, intercept=12.7 mmHg, R²=0.92, P<0.001) or aortic diastolic (slope=1.00, intercept=0.3 mmHg, R²=0.99, P<0.001). Alx had a slope between devices less than unity (slope=0.74, intercept=15.7%, R²=0.36, P<0.001). Taking international guidelines for brachial blood pressure measurement in the absence of guidelines for central aortic blood pressure calculation, the Oscar 2 device has a Grade A rating. However, calculated augmentation index was considerably different between devices. (BP Lab data has been collected, but the analysis is currently unavailable due to a software license issue that will be resolved before the conference date.)

Conclusion: Our findings show that the cuff-based ABPM calculation of aortic systolic and diastolic pressure out of clinic settings appears feasible. However, the calculation of waveform shape parameters, such as augmentation index, requires further investigation.

MUSCLE FITNESS AND BLOOD PRESSURE IN CHILDREN THROUGH TO LATE ADOLESCENCE

Demmer DL°, Belin LJ, Hands B, Burrows S, Straker L, Mori T

°University of Western Australia, Perth, Western Australia, Australia; aInstitute for Heart Health, Notre Dame University, Fremantle, Western Australia; aGraduate School of Health, Perth, Western Australia, Australia

Background: Cardiorespiratory fitness has been shown to reduce mortality rates and attenuate health risks associated with increased adiposity. Muscle strength is an important aspect of physical fitness and low levels of muscle strength are associated with morbidity and mortality outcomes in adults. Muscular mass has been inversely associated with onset of insulin resistance, Type 2 diabetes and high blood pressure (BP) in adults. However, there is a lack of data examining the relationships between muscle strength with BP in childhood and adolescence.

Aim: To examine the association between hand grip strength, back endurance and BP from childhood through to adolescence.

Methods: The study included 1916 participants from the Western Australian Pregnancy Cohort (Raine) Study examined at ages 10, 14 and 17 years. Hierarchical linear mixed model analyses were performed.

Results: The mean hand grip strength in males and females at 10, 14 and 17 years was 30.5 kg, 51.9 kg and 65.5 kg, respectively, and increased over time (P<0.001). Systolic BP increased significantly from 10 years (106.5 mmHg) to 17 years (112.3 mmHg) (P<0.001). Hand grip strength was significantly associated with a higher systolic BP over the 10–17 year age span in both males and females (P<0.001) in regression models that adjusted for gender.
and BMI, but the strength of association was attenuated over time. An increase in hand grip strength of 10 kg was associated with a higher systolic BP of 1.6 mmHg at 10 years and 0.07 mmHg at 17 years. Back endurance was significantly associated with a higher systolic BP at 14 years (P < 0.05) and this association was maintained from 14 to 17 years. Every 100 second increase in back endurance was associated with a 1 mmHg higher systolic BP.

Conclusions: This the first study to examine the relationship between muscle strength and systolic BP from childhood through to late adolescence. The positive association is contrary to that seen in adult populations. However, the attenuation by age 17 suggests a growth-related effect. Hence further studies as the population moves into adult life will be of interest. The underlying mechanism warrants further investigation and the results need to be verified in other young populations.


does concomitant autonomous adrenal cortisol overproduction have the potential to confound the interpretation of adrenal venous sampling in primary aldosteronism?


Endocrine Hypertension Research Centre, University of Queensland School of Medicine, Greenslopes and Princess Alexandra Hospitals, Brisbane, Queensland, Australia

Background: Primary aldosteronism (PA) is a common secondary form of hypertension, with unilateral forms potentially curable by adrenalectomy (ADX). Lateralization of aldosterone (aldo) production by adrenal venous sampling (AVS) is required for ADX. Criteria used are (1) successful cannulation, defined by an adrenal/periadrenal cortisol gradient ≥ 2, (2) aldosterone ratios (AR) for the affected (A) versus contralateral (CL) side > 2, (3) ACR on the contralateral (CL) side < peripheral (CL suppression). We encountered a patient with an aldosterone-producing adenoma (APA) that co-secreted cortisol and in whom AVS failed to lateralize despite subsequent cure of PA following APA removal. We hypothesized that autonomous cortisol secretion confounded the AVS.

Aim: To test the hypothesis that autonomous cortisol secretion confounded AVS by examining hormone levels in patients with isolated cortisol-producing adenoma (CPA) who underwent AVS.

Methods: Eight CPA patients had AVS and Cushing’s syndrome (n=7; 2 “subclinical”), unsuppressible cortisol by dexamethasone (n=8), suppressed ACTH (n=8), unilateral lesion on imaging (n=8) and clinical resolution of hypercortisolism post ADX (n=7; 1 unavaiable). All had normal plasma ald/cort ratios. AVS results were assessed using diagnostic criteria used for lateralization of ald in PA.

Results: Cortisol levels were higher on the ipsilateral (IL) than the CL side of the tumor in all (median 8.7 [range 2.4–27.2] fold; P=0.012), consistent with CPA. By our usual criteria, catheter placement would have been labeled as inadequate CL to the CPA in 6 (75%), pre- sumably because of suppressed cortisol production, despite adrenal vein ald levels being markedly higher than peripheral (median 41.6 [range 7.2–511] fold; P<0.001), suggesting unsuccessful cannulation. In all patients, adrenal vein CL ACTR to the CPA were at least 2-fold higher than peripheral, but in only 3 patients IL to the CPA (median fold difference CL 44.5 [range 6.0–109] vs. IL 1.65 [1.0–23.0]; P=0.017). ACR were higher CL vs. IL in 7 (88%; median 6.0 [range 2.0–76] fold) and similar (19± vs. 23) in the remaining patient.

Conclusion: In patients with PA, concurrent autonomous unilateral hypersecretion of cor- tisol might potentially confound the accuracy of AVS by increasing cortisol levels (reducing ACR) on the side of the CPA, while reducing cortisol levels (increasing ACR and suggesting failed cannulation) on the CL side. Failed lateralization could result in misclassification of AVS. In such patients, use of an alternative (e.g., metadrenaline) to assess success of can- nulation and lateralization should be considered.

the utility of renal vein renin studies in selection of patients with renal artery stenosis for revascularization. a retrospective study


Endocrine Hypertension Research Centre, University of Queensland School of Medicine, Greenslopes and Princess Alexandra Hospitals, Brisbane, Queensland, Australia

Background: Trials failed to demonstrate greater benefit from angioplasty than medical treatment in hypertensive patients with renal artery stenosis (RAS) when patient selection is based solely on radiologic arterial narrowing. All RAS on imaging is not hemodynamically significant and identification of patients likely to benefit from angioplasty remains essen- tial. Renal vein renin studies (RVSs) showing renin production is increased ipsilateral and suppressed contralateral (CL) to RAS are thought by some to predict treatment response.

Aim: To examine whether RVSs performed under highly stringent conditions might assist in predicting favorable blood pressure (BP) outcomes.

Methods: Patients with RAS who underwent RVR between 2008–13 and angio-plasty (+/- stent) were selected for analysis. Drugs known to affect renin levels were replaced before RVR whenever possible. On the 5th day of a <40 mmol/day Na diet in hospital, patients maintained recumbency overnight and during the procedure. Lateralization ratios (RVR) were calculated after stimulation with enalapril/captopril by dividing venous renin levels from the stenotic kidney with CL levels. RVR ≥ 1.5 and CL suppression (CL/peripheral renin levels ≤ 1) were considered positive. "Improvement" was defined as BP ≤140/90 mmHg without medication (“cure”), 10% decrease in mean BP (MBP) without increase of daily defined doses (DDD) of antihypertensive medication or decrease of DDO without 10% increase in MBP Values are expressed as median (interquartile range).

Results: Of 41 patients identified (21 stents), 21 were males, median age 59.1 (49.4, 67.8) years, duration of hypertension 6.0 (1.0, 12.8) years, MBP 104 (98, 110) mmHg, DDO 2.4 (1.5, 4.0) with RAS due to fibromuscular dysplasia (39%) or atherosclerosis (61%). 10 (24%) were positive for DDO and CL suppression ≥ 32%. At follow-up (12.2 [8.1, 14.4] months), 78% of patients were improved (including ≥12% curred). Baseline char- acteristics were similar for improved and unimproved patients with the exception of positive RVR (84% vs. 44%; P=0.025), CL suppression (41% vs. 0%; P=0.038) and chronic kidney disease (CKD: 25% vs. 67%; P=0.042). All patients with CL suppression improved. Improvement was associated with reduction in DDO (–1.0 –1.5, –0.4) vs. 0.3 (0.0, 1.9; P=0.001) rather than change in MBP Logistic regression analysis with RVR and CKD showed that only RVR positivity predicted improved (OR 6.0 (95% CI 1.0–34); P=0.044).

Conclusion: These findings suggest that in selection of patients with RAS for angioplasty, RVRs may help identify those who will benefit.
Moretti JLa, Stevenson ERa, Charchar FJb, Lambert GWb, Davern PLb

From these findings, we suggest that renal sympathetic innervation is essential in maintaining hypertension in BPH/2J mice. The main effect of Rx that occurred equally in BPN/3J and BPH/2J mice was to reduce the basal RAS and SNS-independent contribution to BP. In BPN/3J, BP was maintained by a compensative activation of SNS, whereas there was an increase in RAS contribution in BPH/2J mice. Effectiveness of Rx in reduction of BP may be dependant on the form of hypertension, whether it involves a neuronal or a renal mechanism.

POSSIBLE COMPENSATIVE ACTIVATION OF RAS FOLLOWING RENAL DENERVATION REDUCED BLOOD PRESSURE IN HYPERTENSIVE SCHLAGER MICE

Head GAa, Gueguen Cc, Jackson KLc, Eikelis Nc, Nguyen-Huu Tc, Abegaz Bc, Moretti JLc, Stevenson ERb, Charchar FPb, Lambert GWa, Davern PLc

Aim: To determine whether lower renal miR-181a abundance contributes to elevated RAS activity and hypertension in BPH/2J mice.

Methods: BPH/2J mice (n=8) were administered a miR-181a mimic (miVana, 1, 5, 10 and 25 nmol i.v.) using an in vivo kidney specific transfection reagent and compared with untreated normotensive BPN/3J and BPH/2J mice (n=8–8). Blood pressure (BP) was measured before and for 2 days after mimic treatment via pre-implanted radiotelemetry probes. The BP response to ACE inhibition (enalaprilat) and ganglion blockade (pentolinium) was determined during the dark period – 26 hours after a 25 mm dose and kidney tissue was collected ~50 hours.

Results: The 25 mm dose of the miR-181a mimic caused a 4.0±1.4 mm Hg reduction from baseline in diastolic BP during the dark period (P<0.01), whilst the 1, 5 and 10 mmol doses had no detectable effect (P>0.27). Renal renin mRNA abundance in mice treated with the miR-181a mimic was 0.87±0.1, which was lower than untreated BPH/2J mice (1.5±0.2; P=0.02) and comparable with untreated normotensive BPN/3J control mice (0.95±0.5; P=0.90), suggesting that the mimic effectively inhibited renin mRNA in vivo. Furthermore, the depressor response to enalaprilat in untreated BPH/2J mice was abolished in BPH/2J mice treated with the mimic (~11.2±2 mm Hg vs. 1.5±3 mm Hg respectively; P<0.001), suggesting that the mimic reduced the RAS contribution to BP maintenance. The peak depressor response to pentolinium following enalaprilat pre-treatment was comparable between untreated and mimic treated BPH/2J mice (~5±3 vs. ~5±3 mm Hg; P=0.08), suggesting that the mimic does not overly affect the SNS contribution to BP maintenance.

Conclusion: These findings provide the first in vivo evidence that low miR-181a levels contribute to greater activity of the RAS and hypertension in BPH/2J mice.

OBESITY LIMITS THE NORMAL CARDIAC ADAPTATIONS OF PREGNANCY

Kett MMa, Cai Cc, Cole Jc, Pearson JT

Aim: To examine the impact of obesity on the normal cardiac adaptations of pregnancy and fetal outcomes.

Methods: 4 week old female C5BL/6J mice were fed control (7% fat, 3.85 kCal/g) or high fat (23.5% fat, 5.44 kCal/g) chow for 10 weeks and glucose tolerance tests (i.p.) were performed. Cardiac structure and function were assessed by double-gated cine MRI (4Tesla MR System) prior to mating and at GA14. Fetal and placental tissues were obtained at GA18.5.

Results: High fat feeding generated obese mice with bodyweights 47% greater than controls (33.3±0.6 vs. 22.7±0.2 g; P<0.001). Obese mice had significantly higher fasting blood glucose compared to control mice (9.0±0.25 vs. 6.9±0.37 mmol/L; P<0.001) and were glucose intolerant (P<0.001). Obese mice had significantly greater left ventricle mass (LVM; 67.3±2.1 vs. 58.7±0.9 mg; P=0.01) and cardiac output (CO; 18.3±0.6 vs. 15.6±0.9 ml/min; P=0.05) compared to control mice. Both LVM and CO increased with pregnancy, but the effects were blunted in obese mice (P<0.001; P<0.01). Post-hoc analysis demonstrated that while the pregnancy-induced increases in LVM (~26%) and CO (~25%) for control mice were profound and significant (P<0.001), the increases in LVM (~6%) and CO (~7%) of obese mice with pregnancy were not significant. Indeed at GA14 the values for LVM and CO were no longer different between obese and control mice. The changes in CO with pregnancy were reflected in changes to stroke volume, with only control mice showing a significant increase with pregnancy. The increase in stroke volume in pregnancy control mice was due to increases in end-diastolic volume. Neither obesity nor pregnancy impacted ejection fraction. Litter size at GA18.5 was not different between obese and control dams (7.0±1.2 vs. 7.8±0.7). However, obese dams had significantly greater resorptions and this significantly fewer viable pups (4.0±0.8 vs. 7.5±0.9; P<0.001). Male and female pups from obese dams (1.0±0.2 vs. 0.98±0.02) had significantly lower bodyweights than male and female pups from control dams (0.83±0.04 vs. 0.85±0.04; P=0.01), higher placental weights (P=0.01) and thus lower fetal placenta ratios (P<0.01). Our data suggest that obesity may compromise establishment of effective fetal supply and explain the high rate of miscarriage, stillbirth and small for gestational age babies in obese women.

ARTERIAL HYPERTENSION IN ASTANA CITY, KAZAKHSTAN. A PILOT STUDY

Khamzina Ma, Supiyev Aa, Kossumov Aa, Nurgozhin Ta, Zhumadilov Za, Bobak Mb, Utepova La

Aim: Our data suggest that obesity may compromise establishment of effective fetal supply and explain the high rate of miscarriage, stillbirth and small for gestational age babies in obese women.
Aim: To assess the prevalence, awareness, treatment, and control of arterial hypertension and factors associated with these indices in a population sample of Astana.

Methods: This was a cross-sectional study of subjects registered in 8 outpatient polyclinics in Astana, Kazakhstan.

Participants comprised a total of 497 adults (response rate 56%) aged 50–75 years randomly selected from registers of the polyclinics. Hypertension was defined as a mean systolic and/or diastolic blood pressure of ≥140/90 mmHg and/or anti-hypertensive medication use during the previous 2 weeks. Awareness and treatment were based on self-report. Hypertension control was defined as blood pressure <140/90 mmHg among hypertensive subjects.

Results: The overall prevalence of hypertension was 70%. Among hypertensive subjects, 91% were aware of their condition, 77% were taking anti-hypertensive medications, and 34% had blood pressure controlled (<140/90 mmHg). The prevalence of hypertension and its awareness, treatment and control was more common in women, among persons aged 60 years or more and (except control) among those with high body mass index. None of several available socioeconomic measures was associated with any of the hypertension indices.

Conclusions: The levels of awareness, treatment and control of hypertension in the study group were higher than in most Eastern European and Central Asian populations with available data, most likely reflecting high education and large proportion of civil servants in the new capital city. However, even in this relatively privileged population, the rates of successful control of hypertension were modest.

IMPROVED CARDIAC REPAIR POST-MYOCARDIAL INFARCTION USING NOVEL HUMAN CARDIAC RESIDENT STEM CELLS

Lim SYa, Zhang P, Sivakumar P, Newcomb A, Liu GSb, Elliott DAc, Dusting GJD

1*O’Brien Institute, Melbourne, Victoria, Australia; 2Department of Medicine, University of Melbourne, Melbourne, Victoria, Australia; 3Department of Cardiothoracic Surgery, St Vincent’s Hospital, Melbourne, Victoria, Australia; 4Centre for Eye Research Australia, Melbourne, Victoria, Australia; 5Murdoch Childrens Research Institute, Melbourne, Victoria, Australia

Background: Cardiac stem cells hold much promise for treatment of heart disease, but this remains a nascent field. We have recently identified a novel population of human cardiac resident stem cells (CRSCs), which are positive for W8B2 antigen. W8B2+ CRSCs can self-renew and are highly angiogenic.

Aim: To characterize W8B2+ CRSCs.

Methods: W8B2+ CRSCs were isolated from adult human atrial appendages and studied by various techniques.

Results: Immunophenotyping showed that W8B2+ CRSCs expressed mesenchymal-related antigens but not hematopoietic- or endothelial-related antigens. In the resting state, W8B2+ CRSCs expressed HLA-ABC but not HLA-DR antigen. Regarding cardiac progenitor markers, W8B2+ cells express c-Kit, HAND2, MF2C and Tbx5 but lack expression of c-Ki, Sca-1, NKX2.5, PDGFRα, β1, and Wilms tumor gene 1. This profile is distinct from currently known CRSCs found in the adult human heart, and is further supported by RNA sequencing analysis which revealed many transcripts that were differentially expressed between W8B2+ and c-kit CRSC populations. W8B2+ CRSCs were found to secrete many cytokines implicated in angiogenesis, chemotaxis, inflammation, extracellular matrix remodeling, cell growth and survival. In addition, W8B2+ CRSCs can differentiate into cardiacogenic cells which are responsive to electrical stimulation, as well as into endothelial and smooth muscle cells, and can undergo apolipoprotein, osteogenesis and chondrogenesis. When implanted as cell sheets into an in vivo tissue engineering chamber in rats, W8B2+ CRSCs significantly increased intrinsic vascularization of the engineered tissue constructs. Intramyocardial transplantation of human W8B2+ CRSCs into immunocompromised rats one week after myocardial infarction significantly improved cardiac function (~40% improvement in ejection fraction) and reduced fibrinotic scar tissue. Hearts treated with W8B2+ CRSCs showed less adverse remodeling of the left ventricle, have greater number of proliferating cardiomyocytes (Ki67+ c-Kit+ cells) in the remote region, higher myocardial vascular density, and higher degree of CF163+ cells (a marker for M2 macrophages) infiltration in the border and scar regions.

Conclusions: W8B2+ CRSCs are distinct from currently known CRSCs found in human hearts, and may be an ideal cell source for tissue engineering to treat ischemic heart disease.

INTERRELATIONSHIPS BETWEEN CIRCULATING AND URINARY COMPONENTS OF THE RENIN-ANGIOTENSIN SYSTEM IN INDIGENOUS PREGNANT WOMEN


*School of Biomedical Sciences and Pharmacy, University of Newcastle, Newcastle, New South Wales, Australia; *Mothers and Babies Research Centre, Hunter Medical Research Institute, University of Newcastle, Newcastle, New South Wales, Australia; *Pathology New England, Tamworth, New South Wales, Australia

Background: The renin-angiotensin system (RAS) is activated in pregnancy. In addition, activation of an intra-renal RAS might occur to offset the effects of the high glomerular filtration rate and progesterone.
adaptive immunity and pro-inflammatory phenotype in the kidney, and these pathways may explain mIFN-α1 association with elevation in BP.

**GENETIC VARIATION IN THE RAPTOR GENE IS ASSOCIATED WITH OVERWEIGHT BUT NOT HYPERTENSION IN AMERICAN MEN OF JAPANESE ANCESTRY**

Morris BP*, Carnes BC*, Chen R*, Donlon TA*, He O†, Grove JS†, Masaki KH†, Wilcox BJ†

*Honolulu Heart Program (HHP)/Honolulu-Asia Aging Study (HAAS), Department of Research, Kuakini Medical Center and Department of Geriatric Medicine, John A. Burns School of Medicine, University of Hawaii, Honolulu, Hawaii, USA; †School of Medical Sciences and Bosch Institute, University of Sydney, Sydney, New South Wales, Australia; ‡University of Oklahoma Health Sciences Center, Reynolds Department of Geriatric Medicine, Oklahoma City, Oklahoma, USA

**Background:** The mechanistic target of rapamycin (mTOR) pathway is pivotal for cell growth and has been implicated in aging, cardiovascular disease, obesity, diabetes and cancer. mTOR signalling is involved in cardiac leptin-mediated cardiac hypertrophy and fibrosis associated with obesity. mTOR is a key component of two multiprotein complexes, mTORC1 and mTORC2 complex. mTORC1 complex is pro-growth and contains a unique protein, raptor.

**Aim:** To test, for the first time, whether genetic variation across the raptor gene (RPTOR) is associated with overweight/obesity, essential hypertension (EHT) and isolated systolic hypertension (ISH).

**Methods:** We genotyped 61 common (allele frequency ≥ 0.20) tagging single nucleotide polymorphisms (SNPs) that captured most of the genetic variation across RPTOR in 374 subjects of normal lifespan and 439 subjects with a lifespan exceeding 95 years. Subjects were drawn from the Honolulu Heart Program, a homogeneous population of American men of Japanese ancestry, well characterized for phenotypes relevant to conditions of aging. Hypertension status was ascertained when subjects were 45–65 years old. Statistical evaluation was performed by contingency table analysis, logistic regression and recurrence partitioning (RP), which is regarded as amongst the most powerful methods for statistical analysis of large complex sets of genetic information.

**Results:** After analysis of RPTOR genotypes by each statistical approach we found no significant association between genetic variation in RPTOR and either EHT or ISH. For EHT, RP revealed that even the most predictive SNPs (rs4890052 and rs4969322) provided little contribution to correctly assigning individuals to EHT or MT (P = 0.22 by Z test). In the case of ISH, RP revealed that only one SNP (rs2538118) made a noticeable contribution, and that this was no better than the contribution from the weakest laboratory/examination variable (overweight/obesity). In contrast, for overweight/obesity, the RP model revealed that RPTOR SNPs significantly enhanced the predictive capacity of the model (P = 0.006 by one-tailed Z test).

**Conclusion:** Genetic variation across RPTOR is associated with overweight/obesity, but not EHT or ISH, in American men of Japanese ancestry. (Am J Hypertens Epub ahead of print 22 Sep 2014)

**VASCULAR ENDOThelial CX40 contributes to ACTIVITY-DEPENDENT BLOOD PRESSURE REGULATION**

Morton SK*, Howitt L†, Heisler J*, Nicholson BJ†, Ashton AW†, Mathiassen K†, Hill CE†

*Department of Neuroscience, John Curtin School of Medical Research, Australian National University, Canberra, Australian Capital Territory, Australia; †Department of Biochemistry, University of Texas Health Science Centre, San Antonio, Texas, USA; ‡Division of Perinatal Research, Kolling Institute of Medical Research, Royal North Shore Hospital, University of Sydney, Sydney, New South Wales, Australia; †Department of Molecular Biochemistry, John Curtin School of Medical Research, Australian National University, Canberra, Australian Capital Territory, Australia

**Background:** The role of the gap junction (GJ) protein connexion 40 (Cx40) in renal function has been studied extensively in in vitro and in vivo systems. However, the functional significance of Cx40 in the renal vasculature remains uncertain.

**Aim:** To determine the function of endothelial Cx40 in response to activity.

**Methods:** We isolated cremaster arterioles expressing a mutant Cx40, Cx40T152A, together with native Cx40, under control of the endothelial cell-specific Tie2 promoter. Endothelial Cx40 is essential for ascending vasoconstriction in response to acetylcholine. In vitro, endothelial Cx40 may lead to exercise-induced hypertension.

**Conclusion:** We show that activity-dependent regulation of Cx40 function would lead to dysregulation of blood pressure during activity.

**Hypertension in Zimbabwe: a meta-analysis to quantify its burden**

Mutalw M*, Mangweiro JC*, Lorgelly P†, Owen A†, Renah A†

*Department of Epidemiology and Preventive Medicine, Monash University, Melbourne, Victoria, Australia; †Zimbabwe Diabetes Association, Zimbabwe; ‡Centre for Health Economics, Monash University, Melbourne, Victoria, Australia

**Background:** Hypertension is a recognized global public health problem. Due to a lack of comprehensive national health data, the true magnitude of the problem in Zimbabwe is unknown, and underlying risk factors are not completely understood. Estimating the prevalence of hypertension in Zimbabwe is an important step to informing the development of effective prevention and control strategies.

**Aim:** To estimate the prevalence of hypertension in Zimbabwe and describe its trend since independence in 1980 using secondary source data.

**Methods:** We performed an extensive literature search of MEDLINE, EMBASE and Scopus databases, supplemented by an exploration of bibliographies cited in the articles identified so as to provide further studies. The search was restricted to community or population based studies that used either the WHO (BP ≥ 160/95 mm Hg) or the 7th report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (BP ≥ 140/90 mm Hg) criteria to estimate the prevalence of hypertension in Zimbabwe. The pooled prevalence was calculated using random-effect modeling for meta-analysis. Heterogeneity was assessed using I² statistics.

**Results:** After applying the inclusion and exclusion criteria, a total of 4 studies enrolling 4,829 participants between 1997 and 2010 across 5 provinces in Zimbabwe were identified and included in our review. The pooled prevalence of hypertension in the population was estimated to be 30% (95% CI 19–42). The prevalence of hypertension increased over the 14-year study period (P ≤ 0.005), and appeared to be rising more rapidly in urban settings compared with rural settings.

**Conclusion:** Results from our meta-analysis, of an observed trend towards increasing hypertension prevalence in Zimbabwe, particularly in urban areas, is congruent with other studies documenting increasing hypertension prevalence in less developed countries. Hypertension management in Zimbabwe faces other challenges: economic, environmental and a burden of communicable disease, particularly HIV. Further research is required to understand the reasons behind the increase in prevalence. This will help inform both its management and future prevention. There is an urgent need to develop policies to prevent, detect, treat and manage hypertension effectively in Zimbabwe.

**PRAGMATIC METHOD TO ASSESS TREATED BLOOD PRESSURE FROM HOME BLOOD PRESSURE DIARIES: OPTIMAL STUDY.**

Nelson MR*, Kosmala W†, Blizzard CL†, Sharma J‡

*Menzies Research Institute Tasmania, University of Tasmania, Hobart, Tasmania, Australia; †Murcia Medical University, Wroclaw, Poland

**Background:** Treated blood pressure (HBP) is a better predictor of end-organ damage, cardiovascular events and mortality than clinical measures. HBP is collected and interpreted in general practice in an ad hoc manner. One barrier to using HBP in general practice is the need to calculate average BP.

**Aim:** To develop a timely pragmatic method to assess BP control from patient diaries.

**Methods:** We performed a comprehensive literature search of MEDLINE, EMBASE and Scopus databases, supplemented by an exploration of bibliographies cited in the articles identified so as to provide further studies. The search was restricted to community or population based studies that used either the WHO (BP ≥ 160/95 mm Hg) or the 7th report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (BP ≥ 140/90 mm Hg) criteria to estimate the prevalence of hypertension in Zimbabwe. The pooled prevalence was calculated using random-effect modeling for meta-analysis. Heterogeneity was assessed using I² statistics.

**Results:** After applying the inclusion and exclusion criteria, a total of 4 studies enrolling 4,829 participants between 1997 and 2010 across 5 provinces in Zimbabwe were identified and included in our review. The pooled prevalence of hypertension in the population was estimated to be 30% (95% CI 19–42). The prevalence of hypertension increased over the 14-year study period (P ≤ 0.005), and appeared to be rising more rapidly in urban settings compared with rural settings.

**Conclusion:** Results from our meta-analysis, of an observed trend towards increasing hypertension prevalence in Zimbabwe, particularly in urban areas, is congruent with other studies documenting increasing hypertension prevalence in less developed countries. Hypertension management in Zimbabwe faces other challenges: economic, environmental and a burden of communicable disease, particularly HIV. Further research is required to understand the reasons behind the increase in prevalence. This will help inform both its management and future prevention. There is an urgent need to develop policies to prevent, detect, treat and manage hypertension effectively in Zimbabwe.

**Conclusions:** BP is the most common condition managed in general practice and yet its management is less than ideal. It is usually managed using a clinic derived datum point rather than a community-based dataset that is more representative of true BP. To facilitate uptake of HBP monitoring it is proposed that general practitioners can simply collect and determine a summary statistic by the OPTIMAL method.
A SURGICAL MODEL OF HYPERTENSION AND LEFT VENTRICULAR HYPERTROPHY IN MICE BY SUPRAPARENAL AORTIC CONSTRUCTION.

Nicks AM, Li M, Wu J, Kesteven SH, Fenelly MP, Smith NJ, Ismaa SE, Graham RM

Victor Chang Cardiac Research Institute and University of New South Wales, Sydney, New South Wales, Australia

Background: Surgical models of hypertension and left ventricular hypertrophy (LHV) have been initially developed in larger species and later in the mouse, the latter to meet the demands of transgenic and gene inactivation studies in which mice remain the animal of choice. Surgery, however, is more technically challenging in mice. Supraparenal aortic constriction (SAC) is an approach to inducing high blood pressure as a result of renin-angiotensin-aldosterone-system activation, which is the target of many antihypertensive therapies. Aim: To establish a murine model of SAC and to characterize SAC-induced hypertension and LHV.

Methods: SAC was performed in C57BL/6J mice at 8 weeks of age. The supraparenal aorta (just above both renal arteries) was tied on to a 29 gauge needle with a 7.0 silk suture and the needle was then removed. The diameter of the aorta was estimated to be reduced by approximately one-third of its original diameter. One week post-SAC, micromanometry and echocardiography were performed to determine cardiac function and structure, and tissues were collected for analysis.

Results: Post-SAC, systolic blood pressure increased by 45±2 mmHg (sham 109±2 mmHg, n=19 vs. SAC 154±5 mmHg, n=17; P<0.001), confirming induction of hypertension. Successful constriction, indicated as a systolic blood pressure >2 S.D. above the mean sham value, was achieved in 80%. LHV was evident from an increase in LV mass, enlargement of cardiomyocytes and, consistent with pathological hypertrophy, increased expression of ANP, BNP, β-MHC and α-SMA. Echocardiography showed thickening of the LV wall (h) and reduced LV chamber radius (r), resulting in an increase in the h/r ratio, which also indicates concentric hypertrophy development. This was accompanied by a reduction in cardiac output (~4%, n=7–11; P<0.05), ejection fraction (~8%, n=7–9; P<0.05) and fractional shortening (~11%; n=9–11; P<0.05). Furthermore, one week post-SAC there was evidence of LV remodelling with increased perivascular fibrosis.

Conclusion: SAC-induced hypertension and LHV in mice mimics the phenotype of hypertensive patients with pathological LHV. This surgical model is therefore a valuable research tool for understanding the molecular and biochemical changes underpinning LHV and for evaluating potential therapeutic strategies in wild-type and genetically modified mice.

REINSTATEMENT OF RESPIRATORY SINUS ARRHYTHMIA INCREASES STROKE VOLUME WITHOUT CONCOMITANT INCREASE IN OXYGEN DEBT IN RATS WITH LEFT VENTRICULAR DYSFUNCTION

O’Callaghan El*, Lattoo RM*, Zhao L*, Nogaret A*, Paton JF*

*University of Bristol, Bristol, UK; **University of Sao Paulo, Sao Paulo, Brazil; **University of Bath, Bath, UK

Background: Respiratory sinus arrhythmia (RSA) is the physiological phenomenon whereby heart rate is modulated by respiration. Whilst its physiological function is unknown, our mathematical modelling predicts that RSA saves cardiac energy. Cardiovascular diseases, including heart failure, are associated with a loss of RSA. Aim: To test whether reinstating RSA would improve cardiac function post myocardial infarction (MI) using a novel central pattern generator (CPG) that couples heart rate to respiration.

Methods: Experiments were conducted on Wistar rats (male, 250–300 g) 3 days after left anterior coronary artery ligation (n=7). Rats were anaesthetized with isoflurane (1.2–1.8% in oxygen). The input signal to the CPG was phrenic EMG. The CPG generated output stimulus was delivered to the right cervical vagus nerve. In cohort 1, arterial, heart rate, respiratory rate, expired CO2, body temperature and instantaneous blood flow from the ascending aorta (i.e., stroke volume) were monitored simultaneously and the effect of induced RSA versus tonic bradycardia on cardiac output was tested. In cohort 2, rats were subjected to RSA, tonic vagal nerve stimulation (VNS) or time-matched controls (n=8–10 per group) for 45 min and hearts were freeze-clamp collected and lactate concentration measured for changes in cardiac metabolic function. One-way ANOVA between treatments and post-hoc paired t-tests were performed. Data are presented as means±SEM and P<0.05 was taken as significant.

Results: Cardiac dysfunction was verified by infarct size (~23±3% of left ventricle) and elevated end-diastolic pressures (~16±1 mmHg). Using the CPG, RSA of 15–76 beats per minute (bpm) bradycardia caused a 12±3% increase in stroke volume. RSA amplitude was not correlated with a greater increase in stroke volume (r=0.003). Tonic VNS at matched average heart rate to RSA increased stroke volume by 8±1% (P<0.05 compared to RSA). Despite the increase in stroke volume, no difference in cardiac tissue lactate concentration was observed between RSA, tonic VNS and time-matched control post-MI rats (P>0.05).

Conclusion: Using a novel CPG device, we have demonstrated that, in an acute setting, reinstating RSA improves cardiac function. This may provide a novel therapeutic method of increasing cardiac output without generating an oxygen debt, at least in the rat. Ongoing studies are testing whether cardiac output is further optimized in a large pre-clinical animal model that has a heart rate comparable to that of humans.

IN VITRO CULTURE WITHOUT HUMAN SERUM OR WITH HUMAN SERUM AND MENTHOL DONOR ALTERS CARDIOMYOCYTE ENDOMENT IN POSTNATAL LIFE


*Sansom Institute for Health Research, University of South Australia, Adelaide, South Australia, Australia; **Turrellfield Research Centre, South Australian Research and Development Institute, Adelaide, South Australia, Australia; ***Department of Clinical Medicine, Aarhus University, Aarhus, Denmark

Background: The cardiovascular system is vulnerable to perturbations during pregnancy. Previous studies have shown that an altered intrauterine environment can impact on cardiomyocyte endoment at birth and in early life. Aim: To investigate whether in vitro culture and transfer of the embryo, which are important steps in assisted reproductive technologies and perturbations to the nutritional environment during the periconceptional period, alter cardiomyocyte endoment in postnatal life.

Methods: Embryos were either transferred to an intermediate ewe (ET) or cultured in vitro in the absence of (IC) or presence of human serum (IIVS) and a methyl donor (IVH+M) for 6 days. Naturally mated (NM) ewes acted as controls. At 24 weeks, hearts were collected and slices were sampled using the smooth fractionator technique. The estimation of cardiomyocyte number in the left ventricle was performed using design-unbiased stereological techniques.

Results: ICV and IVH+M groups had an increased number of cardiomyocytes in the left ventricle compared to the NM group, but neither of these groups differed from the NM and ET groups. This effect of treatment was present in only the male (P<0.05) and not the female lambs.

Conclusions: The findings from this study highlight the differential effect of the composition of medium used for embryo culture on cardiomyocyte number in postnatal life. This can have implications for heart health in later life. The increased cardiomyocyte endoment could be due to alterations in processes involved in cardiac remodelling such as apoptosis and autophagy and this requires further investigation.

IMPACT OF IN VITRO EMBRYO CULTURE AND TRANSFER ON BAROREFLEX SENSITIVITY IN EARLY POSTNATAL LIFE

Padhee Ms*, McMillen IC*, Zhang S, Armitage JA*, Head GA*, MacLaughlin SM*, Kleemann DO*, Walker SK*, Morrison JL*

*Early Origins of Adult Health Research Group, Sansom Institute of Health Research, University of South Australia, Adelaide, South Australia, Australia; **School of Medicine, Deakin University, Geelong, Victoria, Australia; ***Neuromodulation Laboratory, Baker ID Heart and Diabetes Institute, Melbourne, Victoria, Australia; ****Turrellfield Research Centre, South Australian Research and Development Institute, Adelaide, South Australia, Australia

Background: Previous studies have highlighted the ability of the nutritional environment of the ovary and early embryo during the periconceptional period to alter blood pressure regulation in fetal and postnatal life. Aim: To investigate whether in vitro embryo culture and transfer, as well as manipulations to the nutritional environment during the periconceptional period, result in dysregulation of baroreflex control of blood pressure.

Methods: Embryos were either transferred to an intermediate ewe (ET) or cultured in vitro in the absence of (ICV) or the presence of human serum (IIVS) and a methyl donor (IVH+M) for 6 days. Controls were naturally mated (NM) ewes. Mean arterial pressure (MAP) and heart rate (HR) were measured using an indwelling carotid artery cannula under basal conditions and during phenylephrine infusion. The relationship between MAP and HR was assessed using the logistic function and the maximal gain was obtained to compare the slopes between different treatment groups. One-way ANOVA was used to analyse the slopes from each curve (SPSS 18 for Windows, Statistical Package for Social Scientists Inc., IL, USA).

Results: Basal MAP and HR were unchanged between the treatment groups. There was no significant difference between the upper plateau (P=0.059), and maximal gain coefficient obtained between the treatment groups (P=0.50) and MAP50 (the pressure at the midrange of the curve) when compared to the NM (P=0.11). However, the males had a higher MAP50 than the females (P<0.05).

Conclusions: The results suggest that embryo transfer and in vitro embryo culture do not alter basal MAP, HR or baroreflex sensitivity.

HIGH BLOOD PRESSURE AS A DRIVER OF STRUCTURAL AND BIOMECHANICAL CHANGES IN MESENTERIC RESISTANCE ARTERIES IN CHRONIC KIDNEY DISEASE

Phillips JK*, Quelk KJ, Boyd R, Amner OZ, Murphy TV*

*Australian School of Advanced Medicine, Macquarie University, Sydney, New South Wales, Australia; **Department of Physiology, School of Medical Sciences, University of New South Wales, Sydney, New South Wales, Australia

Background: Pathological alterations in resistance artery structure and biomechanics are seen commonly in association with hypertension.

Downloaded from http://hyper.ahajournals.org/ by guest on August 30, 2017
Aim: To use pressure myography and histology to investigate mesenteric resistance artery structural stiffness and morphology in a genetic hypertensive rat model of chronic kidney disease (CKD).

Methods: Pressure myography and histology were used to investigate mesenteric resistance artery structural stiffness and morphology in the Lewis polylectic kidney (LPK) rat, at early (12 weeks; n=7) and established (18 weeks; n=7) CKD time-points, relative to age-matched Lewis control rats (n=7 each). Rats were phenotyped for systemic blood pressure (SBP) and urine biochemistry. Rats were then euthanized ( overdose (sodium pentatheline), blood was collected for urea and creatinine analysis, and the mesenteric artery was dissected.

Results: Twelve and 18 week-old LPK exhibited eutrophic and hypertrophic inward remodeling, characterized by increased medial smooth muscle thickness, decreased lumen diameter and unchanged and increased media cross-sectional area (MCSA) in 12 and 18 week-old LPK, respectively, relative to age-matched Lewis controls. Structural changes were not associated with hyperplasia, as no age or strain-related changes in nuclear density or number were found. Larger elastic-modulus/stress slopes (E/σ) and collagen/elastic ratios indicated increased stiffness in LPK at 18 weeks, relative to both age-matched Lewis and 12 weeks LPK (E/σ: 24.2±2 vs. 6.0±0.3; P<0.001; and collagen/elastic ratio: 8.0±2 vs. 2.0±0.5 ±4.1, respectively [P<0.05]). Regression analysis revealed SBP as a main predictor of wall/lumen ratio (r2=0.49; P<0.001). Conclusion: The results indicate that mesenteric resistance arteries in the LPK model of CKD undergo vascular remodelling and stiffness. Hypertension likely predisposes to structural alterations in CKD.

EFFECT OF BLOOD COLLECTION ON BLOOD PRESSURE: IMPLICATIONS FOR ASSESSING CARDIOVASCULAR RISK IN CLINICAL PRACTICE

Picone DS, Cline RE, Sharan JE

Menzies Research Institute Tasmania, University of Tasmania, Hobart, Tasmania, Australia

Background: Doctors may conduct cardiovascular risk assessments that include blood collection and measurement of blood pressure (BP) within the same consultation period. The accuracy of risk assessment could be compromised if blood collection changes BP significantly, but this has not been investigated.

Aim: To determine whether blood collection changes BP significantly.

Methods: Twenty-four participants (56±11 years, 15 male), including 8 patients with type 2 diabetes and 16 healthy nondiabetics, had BP measured (MEG GmbH, Mobil-o-graph) before (x3), during (x1) and after (x3) BP blood collection on the contralateral arm in the seated position to minimize changes in the dimensions of the BP-measuring cuff. The Mobil-o-graph device also provided hemodynamic measures of cardiac output, total vascular resistance and aortic stiffness. On a separate visit, BP was measured in duplicate after 10 minutes rest in the absence of blood collection, as an indication of research-grade clinic BP.

Results: Systolic BP (SBP) measured before, during or after blood collection was not significantly different from research-grade BP (P>0.05 for all, whether diabetic or nondiabetic). There was a trend towards reduced total vascular resistance immediately after blood collection (P=0.01). This was, however, unrelated to changes in BP (P=0.33). Research-grade SBP significantly correlated with SBP measured before, during and after blood collection (intracción correlation coefficient r=0.83; P<0.001 all). Conclusion: SBP measured during blood collection is representative of research-grade BP and does not affect assessment of cardiovascular risk.

FACTORS AFFECTING BLOOD PRESSURE AND RENAL HEALTH IN YOUNG INDIGENOUS PREGNANT WOMEN

Rae V1, Pringle KG2, Sykes S1, Weatherall L1, Claussen D1, Smith RP1, Blackwell CC1, Lumbers EFA1

1Mothers and Babies Research Centre, Hunter Medical Research Institute, Newcastle, New South Wales, Australia; 2School of Biomedical Sciences and Pharmacy, University of Newcastle, Newcastle, New South Wales, Australia; 3Pathology New England, Tamworth, New South Wales, Australia; 4Hunter Medical Research Institute, University of Newcastle, Newcastle, New South Wales, Australia

Background: Indigenous Australians have a high mortality and morbidity from cardiovascular disease and chronic kidney disease.

Aim: To study the health of young Indigenous pregnant women who have participated in the Gomeroi/Gawnggal Arts program.

Methods: Women were aged between 14 and 41 years. Nine, 31 and 60% of the samples were collected in the first, second and third trimesters, respectively and samples were taken from 55, 33 and 11% of women on 1, 2 or 3 occasions. Further data relating to blood pressure was collected from antenatal records.

Results: Only 5 women ever recorded a systolic blood pressure ≥140 mmHg, and 6 women a diastolic blood pressure ≥90 mmHg. On the other hand, 18 women had urinary protein to creatinine ratios ≥30 mg/mmol, 6 had more than one record of proteinuria. Diastolic pressure and plasma creatinin C showed gestational dependence increases (each P<0.001), IgG antibody levels and C reactive protein (CRP) both showed a negative correlation with gestational age (P<0.05). There were positive correlations between systolic pressure and CRP (rho=0.212, P<0.05) and total white blood cell (wbc) count (r=0.322, P=0.017). The correlation between the variables did not reach statistical significance, although there was a negative correlation between cotinine levels (a measure of exposure to cigarette smoke) and diastolic pressure (r=-0.253, P<0.02). Cotinine levels were also directly related to IgG levels (r=0.22, P<0.02) and wbc count (r=0.27, P=0.026) in women with a diastolic pressure >108; P<0.003). In women with a urinary protein/creatinine ≥20 mg/mmol, blood pressures, components of the circulating renin-angiotensin system, and inflammatory markers were similar to those measured in women without proteinuria. However, their albumin/creatinine levels were higher (P<0.001), as were their urinary angiotensinogen, ACE, prorenin and active renin/creatinine ratios (P=0.012, 0.000, 0.021 and 0.002, respectively). Conclusion: In this population of Indigenous Australian women, the incidence of high blood pressure is low, but there is an increased prevalence of proteinuria. Systolic blood pressure appears to be affected by the presence of inflammation, as evidenced by the relationships between systolic pressure, CRP and wbc count. The higher urinary angiotensinogen/creatinine ratio in pregnant women with proteinuria is in accordance with observations made in non-pregnant populations. As well the activity of all components of the intrarenal RAS that were measured was increased in these women, indicative of previously undetected severe renal dysfunction.

UREMIC TOXINS CONTRIBUTE TO CARDIOVASCULAR RISK IN CLINICAL PRACTICE

Rana I1, Kompa AR1, Skommer Ja1, Wang BP1, Lekasavarnit S2, Kelly DJ1, Krum H1, Charchar FP1

1School of Health Sciences, Federation University Australia, Ballarat, Victoria, Australia; 2Centre of Cardiovascular Research and Education in Therapeutics, Monash University, Melbourne, Australia; 3Department of Medicine, University of Melbourne, St. Vincent's Hospital, Fitzroy, Melbourne, Victoria, Australia

Background: A decline in renal function is a common consequence of myocardial infarction (MI) and resulting in increased cardiovascular events, known as cardio renal syndrome (CRS). Although molecular mechanisms contributing to CRS are not well understood, a role for elevated plasma levels of the uremic toxin indoxyl sulphate (IS) and increased fibrosis have been described. MicroRNAs are small endogenously transcribed regulatory RNAs that modulate gene expression and regulate many cardiac processes involved in cardiac dysfunction.

Aim: To investigate, using a rat model, (i) whether MI leads to changes in expression of cardiac microRNA-21 and microRNA-29, both known to contribute to fibrosis and (ii) the effect of lowering plasma uremic toxins on cardiac expression of these microRNAs.

Methods: MI was induced by coronary artery ligation in male Sprague-Dawley rats. At 16 weeks cardiac function was measured prior to sacrifice. Cardiac tissues were assessed for molecular changes using quantitative real-time PCR, western blot analysis and histological methods. Fibrosis was evaluated by calculating the proportional area of picrosirius red stained matrix using image analysis. Cardiac microRNAs-21 and 29b were measured from subtotal nephrectomy rats (STNx) to confirm the role of IS and renal damage. The effect of direct exposure of IS on cultured cardiac fibroblast cells was evaluated.

Results: The percentage area of cardiac fibrosis and delta serum IS levels were significantly higher in the vehicle MI group compared to sham animals (P<0.05 for both). Both were significantly attenuated by oral administration of AST-120, which absorbs toxins in the gastrointestinal tract (P<0.05 for fibrosis and P<0.001 for delta serum IS, compared to MI+Vehicle). MI significantly increased cardiac microRNA-21, collagen 1A1, fibronectin-1 and TG51 mRNA levels, as well as cardiac fibrosis and collagen 1 protein levels. Cardiac microRNA-20 expression was reduced in the heart. Treatment with AST-120 significantly reversed all of these changes. Compared to sham rats, level of cardiac microRNA-21 was increased (P<0.05) and microRNA-29b was decreased (P<0.01) in vehicle treated rats with STNx surgery. This change in microRNA levels was significantly attenuated by AST-120 treatment. Direct exposure to IS was sufficient to increase level of myocardial microRNA-21 and reduce cardiac fibrosis in cultured cells.

Conclusions: We report a link between the beneficial effects of lowering circulating uremic toxins and microRNAs changes in the heart. Targeting microRNAs may provide a therapeutic target for the treatment of CRS.

TARGETING INFLAMMATION IN THE BRAIN DURING ANGIOTENSIN II-INDUCED HYPERTENSION

Ranasinghe NDD1, Dingh QN1, Chu HX1, Chan CT1, Vinh A1, Drummond GR1, Soney CG1, Chrisboils S1

1Department of Pharmacology, Monash University, Clayton, Melbourne, Victoria, Australia

Background: Hypertension is associated with oxidative stress and inflammation, and is a major risk factor for stroke.

Aim: To characterize inflammatory cell types in the brain during angiotensin II (Ang II)- induced hypertension and to test whether deletion of Nox2, a key mediator of inflammation in the brain, attenuates this inflammation.

Methods: C57Bl/6 (wild-type) and Nox2-deficient mice were treated with vehicle (saline) or Ang II (0.7 mg/kg/d, s.c.) for 14 days using osmotic minipumps. Systolic blood pressure (SBP) was measured using tail-cuff plethysmography. Inflammatory cell numbers were measured using flow cytometry and inflammatory cell markers were measured using quantitative real-time PCR.

Results: In wild-type mice treated with Ang II, SBP was elevated (150±4 mmHg, n=20) compared to vehicle-treated mice (118±4 mmHg, n=17; P<0.001). Similarly, Ang II increased SBP in Nox2-deficient mice (157±7 mmHg, n=11) compared with vehicle treated (113±7 mmHg, n=9; P<0.005). Flow cytometric analysis of brain cell suspensions
indicated an increase of microglia (CD45+ CD11b+ F4/80+; 2.1-fold), total leukocytes (CD45+; 3.9-fold) and leukocyte subsets including total microglia (CD45+ Ly6C-; 2.2-fold), neutrophils (CD45+ Ly6G+; 3.9-fold) and T cells (CD45+ CD11b- CD3+; 3.0-fold) in Ang II-infused wild-type mice compared to vehicle (n=12–19; P<0.05). In contrast, there was no difference in any inflammatory cell numbers between Ncx1-deficient mice treated with Ang II or vehicle (n=9–11; P>0.05). Quantitative PCR analysis indicated no effect of Ang II on mRNA expression of interleukin-6, chemokine (C-C motif) receptor 2, and interleukin-10, in the brains of wild-type and knockout mice compared to vehicle (n=12–18; P>0.05).

Conclusions: Ang II induces hypertension that is associated with an increase in several inflammatory cell types in the brain. However, the inflammatory cell infiltration of the brain, but not the hypertension, is dependent on Ncx2 oxidase expression.

PERFORMANCE OF THE BPTRU AUTOMATIC BLOOD PRESSURE MONITOR IN THE ASSESSMENT OF BLOOD PRESSURE CONTROL IN PATIENTS WITH CHRONIC KIDNEY DISEASE

Redzepagic S, Ho FM, Williamson PM, Pirabarhah S, Bedden J, Kelly JP, Ong SLH
*Department of Renal Medicine, St. George Hospital, Sydney, New South Wales, Australia; St George Clinical School, School of Medicine, University of New South Wales, Sydney, New South Wales, Australia

Background: Optimal blood pressure (BP) control is essential in the management of chronic kidney disease (CKD). Although automated BP monitoring (ABPM) provides accurate BP assessment, it may not be readily available. Hence, it is important to identify a simple and accurate BP monitoring device that can be used in the clinic setting.

Aim: To compare the accuracy of the BPTRU device with 24-hour ABPM in diagnosing and monitoring of hypertension in patients with CKD.

Methods: Casual clinic BP was measured in 136 CKD patients using the BPTRU device and a mercury sphygmomanometer. These measurements were followed by 24-hour ABPM. Using the mean 24-hour ABPM as reference standard, the proportions of patients with white coat hypertension (WCH) and masked hypertension (MHT) were determined for readings obtained using the BPTRU and mercury devices. Agreement between the methods was examined by Bland-Altman plots with ABPM as the gold standard. BP data were reported as median with interquartile range.

Results: The median mercury, BPTRU and 24-hour average ambulatory BP readings were 134 (124–148)/74 (67–81), 127 (115–137)/72 (65–79) and 133 (124–142)/72 (66–78) mm Hg, respectively. Systolic BP (SBP) obtained using the BPTRU device was significantly lower than the 24-hour average ambulatory SBP (P<0.005). In contrast, SBP obtained using the mercury sphygmomanometer was higher than the 24-hour average ambulatory SBP (P<0.005). Casual clinic BP measurement using the BPTRU device demonstrated WCH in 32% and sustained HT in 23%. Using a mercury sphygmomanometer, WCH was seen in 2%, MHT in 31%, controlled HT in 30% and sustained HT in 37%. Bland-Altman plot showed good agreement between these BP measurement techniques.

Conclusion: In the clinic setting, BP control in the CKD group was better determined by the mercury sphygmomanometer than the BPTRU monitor. The BPTRU device underestimated SBP and misclassified more cases of true HT as masked HT than a mercury sphygmomanometer.

EPIDEMIOLOGY, HOSPITALIZATION AND HEALTH CARE COSTS FROM HEART FAILURE IN AUSTRALIA: A SYSTEMATIC REVIEW

Sahle B, Owen A, Reid CM
Centre of Cardiovascular Research and Education in Therapeutics, Department of Epidemiology and Preventive Medicine, Monash University, Melbourne, Victoria, Australia

Background: Heart failure is a global public health problem. Its prevalence is projected to rise due to an increase in population aging. Although there are no nationally representative population-based estimates, individual studies have shown that heart failure is a significant cause of morbidity and hospitalization in Australia.

Aim: To conduct a systematic review the literature on epidemiology, hospitalization and health care costs of heart failure in Australia.

Methods: Embase, MEDLINE, PsycINFO and the Australian Institute of Health and Welfare (AIHW) databases were searched for relevant publications on heart failure from Australia between Sep 1980 and Oct 2014.

Results: Twenty-one studies were included in this review. The overall incidence of heart failure was estimated to be 2.1 per 1000, and 5.9 per 1000 person-years in 65 to 84 year-old hypertensive subjects. The prevalence of heart failure was 1.3%, and rose with age up to 7% in those 75 years or older. Hospitalization rates due to heart failure as a primary and secondary diagnosis were 1.8 and 3.4 per 1000 population, respectively. Approximately three-quarters of heart failure patients were readmitted within a year. The mortality rate due to heart failure was 17.1 per 1000,000 person-years and 228 per 1000 in those with known heart failure. The median health care cost per heart failure patient per month was 560 Australian dollars.

Conclusions: There is a significant burden of heart failure in Australia. Further population-level studies on a nationally representative population using objective methods of ascertainment are recommended.

PRESSURE-INDUCED T CELL HOMING IN THE AORTA

Sampson AK, Jennings GJ, Chin-Dusting JPF

Baker Heart and Diabetes Institute, Melbourne, Victoria, Australia

Background: T cell infiltration into target cardiovascular organs, such as the aorta and kidney, is thought to play a role in the etiology of hypertension and disease progression and to promote organ damage. The mechanisms underlying T cell recruitment and infiltration are not known. As we have shown previously high intraluminal pressure can induce endothelial cell activation and cytokine release. We hypothesize that factors released from endothelial cells under pressure can promote T cell recruitment and infiltration.

Aim: To examine the contribution of pressure-induced endothelial cell activation to T cell recruitment.

Methods: T cell migration was assessed in the presence of supernatants collected from human umbilical endothelial cells (HUVECs) that were untreated, treated with TNFα for 24 hours (10 ng/mL), pressed at 120 mm Hg for 24 hours or medium alone with the known migration stimulator, CCL21 (1/100 ng/mL). The number and phenotype of migrated T cells was assessed by antibody-directed flow cytometric analysis. In addition, we examined whether T cell migration was dependent on factors released by the perivascular adiopose tissue (PVAT) surrounding the aorta. PVAT was harvested from the thoracic aorta of 14-week-old normotensive WKY and hypertensive stroke-prone spontaneously hypertensive (SHR-SR) rats and cultured for 24 hours.

Results: The number of migrated T cells in the presence of supernatants collected from untreated cells (773±52, n=6) was lower than supernatants collected from pressed cells (8491±2877, n=4; P<0.01). TNFα-treated cells (36261±1354, n=6; P<0.001) and CCL21 treated medium (23961±1538, n=6; P<0.001) with approximately 48% of migrated T cells in all treatment groups comprising the inflammatory T helper 1 subtype. The migrated T cells in the presence of SHR-SR PVF supernatants comprised a greater proportion of the inflammatory T helper 1 cells compared to WKY PVF supernatants (38.6±2.0% vs. 13.9±2.0%, respectively), despite no difference in the number of total migrated T cells, suggesting that PVAT from hypertensive rats influences T cell recruitment.

Conclusions: We show for the first time that pressure-induced endothelial infiltration and PVAT from chronic hypertensive animals promotes the migration of inflammatory T helper 1 cells, thus providing the first insights into pressure-induced T cell recruitment.

MATERNAL HYPOMAGNESEMIA DOES NOT CAUSE PROGRAMMED CHANGES TO CARDIOVASCULAR PHYSIOLOGY IN ADULT OFFSPRING

Schlegel RN, Moritz KM, Paravincini TM
School of Biomedical Sciences, University of Queensland, Brisbane, Queensland, Australia

Background: Magnesium (Mg) is essential for development and the maintenance of normal cellular processes. Clinical and experimental evidence suggests a strong inverse relationship between Mg deficiency and the development of cardiovascular disease. It is also well established that maternal perturbations such as dietary nutrient deficiencies can result in an increased susceptibility to disease in offspring, including high blood pressure. However, few studies have explored the implications of maternal Mg deficiency and programmed cardiovascular outcomes in offspring.

Aim: To establish a model of Mg deficiency prior to and during pregnancy using CD1 female mice and investigate cardiovascular outcomes in adult offspring at 6 months of age.

Methods: Female CD1 mice (7 weeks old) received either a control Mg diet (0.2% w/w Mg), or Mg deficient diet (MDD) (0.02% w/w). Mice were maintained on this diet for 4 weeks prior to mating and throughout gestation. After 4 weeks on the diet, mice were mated overnight with age-matched CD1 males. Mice were allowed to litter naturally and offspring were reared to age 6 months for analyses. Offspring were implanted with radiotelemetry probes to measure blood pressure. Stress tests were conducted following probe implantation to measure cardiovascular reactivity (changes in mean arterial pressure and heart rate during stress). These included a physical stressor (restraint stress), a non-physical stressor (dirty cage swap), and a “positive stressor” (almond feeding test).

Results: Offspring were born growth restricted, but underwent catch-up growth after weaning. We found no change in basal mean arterial pressure or heart rate in adult offspring. Tests for cardiovascular reactivity showed no change in mean arterial pressure or heart rate between groups or sexes.

Conclusion: Despite evidence suggesting that poor maternal nutrition and fetal growth restriction can program hypertension in adult offspring, our findings demonstrate that maternal Mg deficiency does not cause programmed changes in basal cardiovascular physiology or cardiovascular reactivity to stress in adult offspring.

ARTERIAL IMPEDANCE MISMATCH FAILS TO EXPLAIN CENTRAL PRESSURE AUGMENTATION: AORTIC RESERVOIR FUNCTION MAY BE THE PREVAILING FACTOR

Shultz MG*, Davies JB, Hughes AD, Sharman JB
*Menzies Research Institute Tasmania, University of Tasmania, Hobart, Tasmania, Australia; International Centre for Circulatory Health, Imperial College London, London, UK; *Institute for Cardiovascular Science, University College London, London, UK

Background: Central augmentation pressure (AP) and augmentation index (AIx) are believed to occur due to wave reflection from central-to-peripheral arterial mismatch. High-impedance from stiffer peripheral vasculature should cause increased wave reflection, AP and AIx. Recent evidence suggests, however, that aortic reservoir function may be a more dominant contributor to AP and AIx.
Aims: To determine (i) the relationship of central-to-peripheral arterial impedance mismatch with PA and Aix, and (ii) independent correlates of central BP augmentation.

Methods: Carotid-to-femoral (aortic) pulse wave velocity (aPWV) and carotid-to-brachial PWV (bPWV) were measured in 359 individuals (aged 61±9, 49% male). Central PA, Aix and aortic reservoir pressure were derived from radial tonometry. Participants were stratified according to high- (hPWV > aPWV, n=118) or low-impedance mismatch (hPWV ≤ aPWV, n=241).

Results: Central AP and Aix were significantly higher in participants with low-impedance mismatch compared to those with high-impedance mismatch (11.6±6.8 mm Hg; P<0.001 and 24±11 vs. 21±13 %; P=0.01). Impedance mismatch (bPWV-aPWV) was weakly and negatively associated with PA (r=-0.17; P=0.001), and was not associated with Aix (r=-0.05; P=0.31). Conversely, aortic reservoir pressure was a strong correlate of Aix (r=0.58; P<0.001) and PA (r=0.77; P<0.001). In multivariable linear regression, reservoir pressure predicted PA and Aix independent of age, sex, systemic vascular resistance, heart rate, mean arterial pressure and height (standardized β=0.45 and 0.19; P<0.001 for both, respectively).

Conclusions: Conflicting with conventional theoretical expectations, high-impedance mismatch between central and peripheral arteries does not cause higher AP or Aix. Aortic reservoir function, rather than discrete wave-reflection from arterial impedance mismatching may better explain PA and Aix.

THE EFFECT OF HIGH INTRALUMINAL PRESSURE ON EndoMT-INDUCED FIBROSIS

Shihata WA, Sampson AK, Khammy O, Horlock D, Kaye DM, Chin-Dusting JPF

Background: High intraluminal pressure (HfIP), a proposed explanation (HFPEF) accounts for ~50% of heart failure cases. Patients with HFPEF do not respond to current heart failure treatment therapies and the mechanisms leading to HFPEF are unknown. Critically, HFPEF patients display increased cardiac fibrosis compared to non-HFPEF patients. Endothelial-to-mesenchymal transition (EndoMT) is a major contributor to the development of cardiac fibrosis, but its initiating factors remain unknown. One major risk factor implicated in the development of HFPEF is hypertension, which has also been shown to promote endothelial activation and vascular inflammation, both of which are observed in HFPEF patients and are essential for EndoMT. We hypothesize that high blood pressure per se promotes EndoMT.

Aim: To examine whether high intraluminal pressure contributes to EndoMT-induced fibrosis.

Methods: We examined endothelial to fibroblast transition in human umbilical venous endothelial cells (HUVECs) after 1, 3 and 5 days of treatment. mRNA expression of fibroblast and endothelial genes was assessed by real-time PCR in HUVECs untreated or treated with TGFβ1 (10 ng/ml), TGFβ2 (10 ng/ml) or pressurized to 120 mm Hg. We observed consistently greater expression of the fibrogenic genes vimentin and alpha-smooth muscle actin (αSMA), in the TGFβ1 and 2 treated HUVECs compared to the untreated cells, particularly at 3 days when TGFβ1-treated cells expressed significantly greater vimentin mRNA levels compared to untreated cells (>2 fold change). Concurrently, expression of the endothelial gene, CD31, was consistently reduced in the TGFβ1-treated cells compared to the control group (>2 fold change) suggesting that these cells were undergoing transition away from an endothelial and towards a fibroblast phenotype. HUVECs exposed to 120 mm Hg over 1, 3 and 5 days elicited a consistently lower CD31 gene expression compared to unpressurized cells. Conversely, both vimentin and αSMA mRNA expression in the pressurized cells relative to the unpressurized cells at 5 days (>3 fold change in vimentin; >2 fold change in αSMA), suggesting that exposure to increased pressure promotes transition of endothelial cells towards a fibroblast phenotype.

Conclusions: High intraluminal pressure per se induces EndoMT, observed by a reduction in endothelial expression profiles and an increase in a mesenchymal/fibroblast phenotype. The study suggests that hypertension-induced EndoMT may contribute to cardiac fibrosis and may provide a novel therapeutic target to prevent development of fibrosis in HFPEF.

THE EFFECTS OF POSITIVE ALLOSTERIC MODULATION OF GABAA RECEPTORS ON STRESS AND HYPERTENSION IN SCLERANGiocardial hypertrophic mice

Stevenson ERa, Johns EMCb, Marques FZc, Jackson KL, Evans RGa, Davern PJa, Head GAa

Background: Coronary reperfusion is the primary treatment for a heart attack to reduce infarct size but the reperfusion injury, which causes myocyte death, can be reduced by prior transient ischemia, which induces remote ischemic preconditioning (RIPC) that further reduces infarct size. The mechanisms by which RIPC causes cardioprotection are unclear, but are thought to involve neural and humoral mechanisms.

Aim: To determine the role of vagal innervation in determining the cardioprotective effect of RIPC.

Methods: Anesthetized sheep were allocated randomly into 4 groups: (i) Sham conditioning (n=6), (ii) RIPC conditioning (n=6), (iii) Proximal vagal denervation + RIPC (n=6) and (iv) Distal vagal denervation + RIPC (n=8). RIPC involved 3×5 min occlusions of the iliac artery. Subsequently, the animals underwent 1 hour coronary ischemia followed by 3 hour reperfusion. Hemodynamic parameters were measured throughout the protocol. At the end of 3 hour reperfusion the heart mass and infarct size was measured.

Results: There were no clinically relevant significant differences in hemodynamic parameters among the 4 treatment groups. Compared with Sham animals, RIPC significantly reduced infarct size following IR (infarct size/area-at-risk, 82±5% vs. 49±7%, respectively;
ROLE OF THE ANGIOTENSIN TYPE 2 ON MACROPHAGE FUNCTION AND PHENOTYPE

Wing A, Krishman SM, Wei Z, Deep H, Widdop RE, Vinh A

Department of Pharmacology, Monash University, Melbourne, Victoria, Australia

Background: The pro-inflammatory actions of macrophages are well known to contribute to cardiovascular disease. Macrophages also express components of the renin-angiotensin system, including the anti-inflammatory angiotensin type 2 (AT2) receptor (AT2R).

Aim: To investigate the role of direct AT2R stimulation on macrophage cytokine production and phenotype.

Methods: To induce cytokine production, RAW264.7 and bone marrow-derived macrophage (BMDM) cell lines were stimulated with lipopolysaccharide (LPS) (10 ng/mL) for 6 hours. Treatment groups included an unstimulated control, and LPS in the presence of vehicle, an AT2R peptide agonist, CGP42112 (10−10 μM), an AT2R nonpeptide agonist, compound 212 (212: 0.1–10 μM). Pro-inflammatory mediators (TNF-α, CCL2 and IL-6), regulatory cytokine IL-10 and M1/M2 polarization were measured in treated macrophages using flow cytometry. Quantitative analysis of cytokine secretion was performed using a cytometric bead array.

Results: The proportion of LPS-induced TNF-α, IL-6 and CCL2-producing BMDMs was significantly reduced by ~3% (n=14; P<0.05), ~23% (n=12; P<0.01) and ~23% (n=7; P<0.05), respectively, when co-treated with peptide AT2R agonist, CGP42112. Consistent with these reductions, C212 also significantly reduced TNF-α (~20%; n=14; P<0.01), IL-6 (~18%; n=12; P<0.01) and CCL2-production (~24%; n=7; P<0.05) in BMDMs. C212 was also effective at inhibiting the proportion of TNF-α (~15%; n=13; P<0.001) and CCL2-production (~27%; n=6; P<0.05) in RAW264.7 macrophages. LPS also increased IL-10-producing cells in both RAW264.7 (~38%; n=6; P<0.05) and BMDM (~50%; n=11; P<0.01), which was specifically augmented with compound 212 (~72%; P<0.001).

Conclusions: Our results indicate that direct AT2R stimulation inhibits LPS-induced pro-inflammatory cytokine production, which may be associated with a modest increase in IL-10 production. Therefore, the AT2R represents a potential therapeutic target to control macrophage-dependent inflammation associated with cardiovascular disease.

DOES INCREASING MATERNAL INSULIN SENSITIVITY DURING THE PERICONCEPTIONAL PERIOD CORRECT THE CARDIAC DEFICITS INDUCED BY INTRAUTERINE GROWTH RESTRICTION IN THE SHEEP FETUS?

Zhang S, Harding JE, Padhee M, Botting KJ, Dunn SL, Brooks DA, McMillen IC, Morrison JL

Division of Health Sciences, University of South Australia. Adelaide, South Australia, Australia

Background: Intrauterine growth restricted (IUGR) fetuses have fewer cardiomyocytes and a susceptibility to cardiac hypertrophy and thus ischemic heart disease in adult life. Fetal adaptations to IUGR impact on the abundance of molecules that regulate cardiac growth and metabolism. Few studies have attempted to correct these cardiac deficits.

Aim: To intervene in pregnancies at a risk of IUGR by increasing maternal insulin sensitivity during the periconceptional period and investigate the expression of genes which regulate cardiac growth and development in the late gestation sheep fetus.

Methods: Non-pregnant ewes underwent a carunclectomy to induce placental restriction (PR) and IUGR. In these Control and PR ewes (factor 1: treatment), miniosmotic pumps were inserted subcutaneously one week before mating to deliver either vehicle or rosiglitazone (8 mg/day; factor 2: maternal periconceptional drug exposure) and removed 6 days after mating, resulting in 4 groups. Samples of the left ventricle were collected at 140 days gestation (term, 150 days) and specific mRNAs were measured by quantitative real-time PCR. Data were analysed using a 2-way ANOVA (factors: treatment and maternal periconceptional drug exposure) using SPSS.

Results: PR fetuses were smaller than Controls regardless of periconceptional exposure to vehicle or rosiglitazone. The levels of mRNAs encoding key molecules involved in the cell cycle (SPAG5, BIRC5, CCDC2), glucose metabolism (PKD-2), fatty acid metabolism (PPAR-γ, PGC-1α, PPAR-γ, PPAR-δ), and apoptosis (BCL-2) were increased in PR fetuses compared to Controls, while mRNA encoding the cell cycle inhibitor p21 was decreased, regardless of maternal periconceptional exposure to vehicle or rosiglitazone. Maternal rosiglitazone exposure during the periconceptional period increased mRNA encoding LC3B, a marker of autophagy, regardless of treatment. PR fetuses whose mothers were exposed to rosiglitazone in the periconceptional period had higher AMP mRNA compared to Control fetuses with maternal periconceptional rosiglitazone exposure or PR fetuses with maternal periconceptional drug exposure.

Conclusion: Placental restriction caused a decrease in the cardiac expression of mRNAs encoding molecules involved in proliferation in late gestation, along with a shift in the levels of mRNAs encoding metabolic enzymes that may favour fatty acid metabolism over glucose metabolism. Increasing maternal insulin sensitivity during the periconceptional period did not correct the cardiac effects of PR measured in this study, and may impose additional deficits, but only in the IUGR fetus.

HEMODYNAMIC CHARACTERIZATION OF 300 DAY-OLD NOD B10 FOZ/FOZ MICE

Zhang Y, Arnolda L

Department of Pharmacology, Monash University, Melbourne, Victoria, Australia
Introduction: Obesity and diabetes are major risk factors for heart attack and are associated with hypertension, cardiac hypertrophy and cardiomyopathy. The present study investigated hemodynamic parameters and cardiac weight of fat Aussie (foz/foz) mice (obese and diabetic) studied on a mixed (NOD and B10) background.

Methods: Blood pressure (BP) and heart rate (HR) were measured by tail-cuff and carotid artery catheterization in 300 day-old male and female foz/foz and age-matched wild-type (WT) mice (n=10–11 in each group). Cardiac size and function were measured by echocardiogram. Results were expressed as mean±SEM.

Results: BP was higher while HR was lower in foz/foz mice than WT. This trend was consistent in tail cuff and carotid artery catheterization. Left ventricular (LV) diastolic pressure was similar in each group. LV dP/dt, a marker for cardiac contractility, was increased in foz/foz mice compared to WT in female mice. LV end-diastolic diameter and cardiac output (CO) were increased in foz/foz mice. LV posterior wall was thicker in male (1.16±0.04 vs. 0.98±0.06 mm) and septum was thicker in female foz/foz mice than WT (1.21±0.06 vs. 0.91±0.04 mm). Left ventricle+septum weight (LV+S)/tibia length was higher in foz/foz mice than WT mice. BW, LV+S/tibia, SBP, LV dP/dt diameter, CO (g, mg/cm, mm Hg, mg Hg/s, mm, ml/min, respectively) were male: WT: 39.4±1.1, 57.2±1.9, 117±3, 1028±1390, 117+0.14, 17.3±1.1; foz/foz: 60.3±1.4,* 78.1±2.9,* 131±3,* 125±1354, 14.18±0.11,* 26.1±1.1,* female: WT: 33.0±2.0, 39.6±1.5, 109±2, 6673±931, 3.37±0.08, 18.9±1.4; foz/foz: 56.6±2.3,* 60.4±2.8,* 129±4,* 10047±653,* 4.07±0.22,* 21.9±0.5,* where * indicates P<0.05 vs. WT (unpaired t-test)

Conclusion: foz/foz mice develop hypertension, cardiac enlargement and cardiac hypertrophy, together with increased cardiac contractility, indicating that this is a good model for cardiovascular complications of diabetes.