Abstracts From the
37th Annual
Scientific Meeting of
the High Blood Pressure Research
Council of Australia

Melbourne, Australia
December 2-4, 2015
Editor: Brian J. Morris
HBPRCA Oral Presentations

COULD THE PHASE OF THE MENSTRUAL CYCLE AFFECT THE RESULTS OF ADRENALEN VENOUS SAMPLING AND SUBTYPING OF PRIMARY ALDOSTERONISM?

*Endocrine Hypertension Research Centre, University of Queensland School of Medicine, Greenslopes and Princess Alexandra Hospital, Brisbane; *Sullivan & Nicolaides Pathology, Brisbane, Australia

Background: Since aldosterone and cortisol levels vary during the menstrual cycle, and are utilized in the interpretation of adrenal venous sampling (AVS) to differentiate unilateral from bilateral primary aldosteronism, outcome of AVS could possibly be influenced by the time of sample collection.

Aim: To assess the effect of the phase of the menstrual cycle on adrenal and peripheral venous aldosterone and cortisol levels and on serum female sex steroid levels during AVS.

Methods: In 23 pre-menopausal women with primary aldosteronism undergoing AVS, levels of aldosterone, cortisol, progesterone, estradiol, LH and FSH were compared, noting whether: To investigate the effect of follicular or luteal phase. Results were compared to those in age-matched males undergoing AVS.

Results: Based on AVS results, 10 women (7 sampled during luteal phase) had unilateral over-production of aldosterone, and 13 (10 sampled during follicular phase) had bilateral over-production of aldosterone. The difference in proportions of luteal vs. follicular studies that showed unilateral disease was significant (P<0.05). Simultaneously collected peripheral levels of progesterone (P<0.001), estradiol (P<0.05) and aldosterone (P<0.05) and bilateral adrenal venous levels of aldosterone (P<0.01) and cortisol (P<0.01) were higher in those sampled during the luteal phase. Importantly, luteal higher/lower side AV aldosterone/cortisol ratios were higher than follicular (P<0.05). Peripheral and adrenal venous aldosterone and cortisol levels and higher (but not lower) side AV aldosterone/cortisol ratios were significantly higher than male in the luteal group, but not the follicular.

Conclusion: The phase of the menstrual cycle during which samples are collected significantly affects levels of aldosterone and cortisol used to interpret AVS. A risk of false lateralization appears to be present when AVS is performed during the luteal phase, but this requires confirmation with larger patient numbers, preferably with studies in each patient during both phases. Meanwhile, recording of the phase of the cycle during which AVS is performed should be encouraged.

DRINKING 1% SALINE CAUSES HYPERTENSION IN STREPTOZOCIN-TREATED RATS VIA ACTIVATION OF MICROGLIA IN CENTRAL CARDIOVASCULAR CONTROL CENTRES

Alahmadi E, Badoer E, Woodman OL, Stebbing MJ
*School of Medical Sciences and Health Innovations Research Institute, RMIT University, Melbourne, Victoria, Australia; *Department of Pathology, Taibah University, Medina, Saudi Arabia

Background: We have previously reported that streptozocin (STZ)-treated hyperglycemic rats display activation of microglia, the brain’s local inflammatory cells, within the paraventricular nucleus (PVN) and other cardiovascular centers 6-8 weeks following induction of diabetes. The microglial activation was accompanied by intense neuronal activation as well as signs of dehydration, including increased plasma osmolality. Studies in other animal models suggest activation of microglia within the PVN can cause hypertension. By contrast, most studies on STZ rats have observed either reduced or unchanged blood pressure, but this requires confirmation with larger patient numbers, preferably with studies in each patient during both phases. Meanwhile, recording of the phase of the cycle during which AVS is performed should be encouraged.

Methods: Blood parameters, conscious blood pressure (via tail cuff) and baroreceptor sensitivity under urethane anesthesia were measured in control and STZ-treated rats and the effects of substituting 1% saline for drinking water and infusing the drug minocycline into the brain via an osmotic pump were determined.

Results: STZ-treated rats given saline for 2 weeks showed reduced signs of dehydration (P<0.05 for both plasma osmolality and hemoglobin concentration), but also displayed greatly increased microglial activation within the PVN and NTS (P<0.001), whereas control rats given water or saline and STZ-treated rats given water to drink showed no signs of microglial activation at this time point. STZ-treated rats drinking saline also showed significantly increased blood pressure (systolic 132 mmHg, vs control 40 mmHg; P<0.001) and decreased baroreceptor sensitivity (P<0.01) in comparison to both control rats and STZ-treated rats given tap water. When the drug minocycline was infused directly into the brain to inhibit microglial activation, the hypertension seen in STZ-treated rats drinking saline was prevented (P<0.001), strongly suggesting that activation of these inflammatory cells plays a role in generating the increased blood pressure observed.

Conclusion: While STZ-treated rats show dehydration and potentially reduced blood volume, a treatment that prevented dehydration also reduced baroreceptor sensitivity and caused brain inflammation leading to hypertension. Our results demonstrate a novel mechanism by which salt intake may normalize hydration state, yet contribute to hypertension in diabetes.

ASSESSMENT OF RELIABILITY OF HOME BLOOD PRESSURE MONITORING IN CHRONIC KIDNEY DISEASE PATIENTS

Amer ZS, Ong SLH, Kelly JP
*Department of Renal Medicine, St George Hospital, Sydney, New South Wales, Australia; *St George and Sutherland Clinical School, University of New South Wales, Sydney; New South Wales, Australia

Background: Home blood pressure monitoring (HBPM) is a valuable component of monitoring essential hypertension. There are limited data, however, on its role in chronic kidney disease (CKD).

Aim: To assess (i) the reliability of the current HBPM protocol and (ii) to identify barriers to patient compliance in those with CKD.

Methods: 95 CKD clinic patients undertook HBPM following a predetermined HBP protocol. OMRON BP monitors (HBP monitor HEM 7211) were used after calibration of monitors on each patient in the clinics. Patients were asked to check BP three times, both in the morning and evening for seven days, record their measurements and then calculate BP averages. Patients were randomized into a group who were told their readings would be audited (audit aware) and a group who would not be audited (audit unaware). Patient reported readings were compared against monitor-recorded memory and patient calculated blood pressure averages were checked for accuracy.

Results: The group comprised of 59% males and 41% females, whose ages ranged from 25 to 98 years and whose mean estimated glomerular filtration rate (eGFR) was 47.5 mL/min (range 9 to 80 mL/min). Twenty four percent of patients had eGFR ≥60 mL/min; 48% had eGFR 30–59 mL/min and 28% had eGFR <29 mL/min. At calibration in the clinic, there was no significant difference in systolic readings obtained by clinic or home monitors, the mean difference in systolic blood pressure being 3.5 mmHg lower (95% CI -8.0 to 0.94; NS) in the HBPM device group. During the week of HBPM, 86% of audit-aware patients completed 7 days of HBPM but only 48% followed the detailed protocol instructions precisely. Of audit-unaware subjects, 84% completed 7 days of HBPM, with only 25% following the protocol. The main protocol violations were failure to calculate BP averages (45%), extra readings (41%), and non-verifiable BP readings (11%). Clinic measurements suggested that 47% of the patients had controlled BP (systolic <140 mmHg), whereas HBPM suggested that 37% of the patients had controlled BP (systolic <135 mmHg; χ²=4.0; P=0.04; NS).

Conclusion: The present study indicates suboptimal compliance with the current standardized HBPM protocol in a CKD population. Impediments to compliance included the high number of readings required, transcription of readings from the HBPM to the written BP diary and the difficulty CKD patients had in the calculation of average BP. There was no significant difference in the assessment of BP control between clinic and HBP readings. Further evaluation of the role of HBPM in the management of CKD is required. Modification of existing protocols to improve acquisition of an accurate HBPM record should be considered.

FACTORS ASSOCIATED WITH AWARENESS, TREATMENT AND CONTROL OF HYPERTENSION IN A RURAL SOUTH INDIAN POPULATION

Busingye D, Arabshahi S, Evans RG, Srikanth VK, Kartik K, Kalyanram K, Riddell MM, Zhu Y, Suresh OP, Thrift AD
*Department of Medicine, School of Clinical Sciences at Monash Health, Monash University, Melbourne, Victoria, Australia; *Cardiovascular Disease Program, Biomedicine Discovery Institute and Department of Physiology, Monash University, Melbourne, Victoria, Australia; *Rush Valley Rural Health Centre, Rich Valley, Andhra Pradesh, India; *School of Earth, Atmosphere and Environment, Monash University, Melbourne, Victoria, Australia; *Forey Institute of Neuroscience and Mental Health, Melbourne, Victoria, Australia

Background: Hypertension is rapidly becoming a major public health burden in rural Indian populations but awareness, treatment, and control are poor in these settings.

Aim: To identify factors associated with awareness, treatment, and control of hypertension.

Methods: Following screening of the population, individuals with hypertension (blood pressure ≥140/90 mmHg or taking antihypertensive medications) were invited to participate in more comprehensive assessments. During these assessments, BP, height, weight,
BLOOD PRESSURE MANAGEMENT – ISOMETRIC HANDGRIP EXERCISE REDUCES HYPERTENSION
Carlson DP, Inger J, McFarlane JR*; Dieberg G; Smart NA*

*University of New England, Armidale, New South Wales, Australia

Background: Hypertension is responsible for 45% of cardiovascular deaths due to heart disease and 51% due to stroke worldwide (WHO). According to the Australian Bureau of Statistics, 31.6% of the Australian population had hypertension in 2011/2012. Our recent meta-analysis indicates that isometric exercise may be an effective treatment for those unable to conduct the recommended minimum 30 minutes per day of moderate aerobic exercise. The anti-hypertensive effect threshold of isometric handgrip training has not been established. Moreover, the usual handgrip intensity of 30% maximum voluntary contraction (MVC) used in most studies is initially challenging for some people.

Aims: To investigate the isometric handgrip intensity threshold for an anti-hypertensive effect, and the possibility of using a 5% MVC group as either a low intensity effect group or a true working control.

Methods: A randomized trial was conducted of 24 participants, aged between 30 and 70 years, diagnosed with mild or pre hypertension, men (n=9) and women (n=15), aged 51±8.2 years. Adherence to training was 100%. Groups were matched at baseline for age, gender, systolic blood pressure (SBP) and diastolic blood pressure (DBP). There were no reported changes in exercise, diet and medication throughout the study for any of the participants. Participants had a resting SBP ≤120 mmHg and/or a resting DBP ≤80 mmHg, or receiving an anti-hypertensive medication (71%). Participants trained 3 days per week for 8 weeks using a BHD-3 Digital Hand Dynamometer with their non-dominant hand, at either 5% or 30% of their MVC.

Participants completed 4 sets of 2-minute isometric handgrip contractions separated by 3-minute rest periods. During one weekly training session resting and handgrip blood pressure was continuously recorded so that fluctuations of single measurements could be avoided. Data were analyzed using paired t-tests and two-way ANOVA in R (version 3.1.3).

Results: In the 30% MVC group, a significant reduction in SBP of ~10 mmHg, from 133.6±4.4 to 123.7±2.3 mmHg (P<0.007), was seen, while in the 5% MVC group a reduction of ~5 mmHg, from 125±1.7 to 120±1.5 mmHg (P=0.03), was noted. Reductions in DBP in the 30% and 5% MVC groups were ~4 mmHg, from 75±5.1 to 71±7.6 (P=0.07), and ~6 mmHg, from 47±8.6 to 46±8.9 (P=0.05), respectively.

Conclusions: The significant reduction in SBP in the 30% group and DBP in the 5% MVC group confirms previous findings. While reductions in SBP in the 5% group and DBP in the 30% group were not significant, they both indicated trends towards blood pressure reduction, particularly for the latter group. Our results suggest that 5% may be a suitable introductory intensity to achieve anti-hypertensive effects in people unable to begin at the desired 30% MVC intensity. Further studies, increasing the number of participants is required to clarify the efficacy of 5% MVC intensity.

ROLE OF INFLATION, VASOCONSTRICITION AND OXIDATIVE STRESS IN THE ENHANCED PRESSOR RESPONSE TO ANGIOTENSIN II IN AGED MICE

*Cardiovascular Disease Program, Biomedicine Discovery Institute and Department of Pharmacology, Monash University, Clayton, Victoria, Australia; The Institute for Molecular Bioscience, The University of Queensland, Brisbane, Queensland, Australia; Current affiliation: College of Pharmacy, Ohio Northern University, Ohio, USA

Background: The prevalence of hypertension increases with age. Chronic low-grade inflammation commonly occurs with aging, and inflammatory mediators are mediators of cardiovascular responses. We tested whether aged mice exhibit an enhanced pressor response to angiotensin II (Ang II) and whether this is associated with inflammation, enhanced vaso-constriction and vascular oxidative stress. We also tested the effect of MCC950, a NLRP3 inflammasome inhibitor, on blood pressure (BP) in Ang II-treated aged mice.

Methods: Young (8–12 week old) and aged (24–30 month old) male C57BL/6 mice were left untreated, or treated with either vehicle or a “slow-pressor” dose of Ang II (0.28 mg/kg) for 28 days. Another group of aged mice were treated with either Ang II + saline or Ang II + MAr950 (10 mg/kg) for 10 days. We measured systolic BP; mRNA expression of inflammatory markers and components of the renin-angiotensin system, vascular contractile responses and superoxide levels.

Results: In young mice, Ang II caused a gradual increase in BP (from 108±5 to 142±8 mmHg; n=8), whereas the effect was much greater in aged mice (from 112±4 to 155±12 mmHg; n=9; P<0.05). Aging alone increased renal expression of AT1a receptors, NLRP3, caspase-1, IL-1β, IL-18, CRCL and CCL5 by >1.5-fold (n=7–8; all P<0.05). Maximum contractile responses to Ang II in mesenteric arteries were selectively enhanced (by 1.8-fold) in aged vs. young mice (n=4; P<0.05). In aged mice, contractile responses to Ang II were not affected by acute pre-treatment with the nitric oxide synthase inhibitor L-NAME (100 µmol/L; n=4) or the cyclo-oxygenase inhibitor indomethacin (3 µmol/L; n=3), but were reduced by the superoxide scavenger tempol by 1.3-fold (100 µmol/L; n=3; P<0.05). Aged mice exhibited increased NADPH-dependent superoxide production in mesenteric arteries (by 2.4-fold) and thoracic aorta (by 2-fold) compared to young mice (n=8–10; both P<0.05). Ang II-induced BP was unaffected by MCC950 vs. vehicle in aged mice (BP: 193±7 vs. 145±10 mmHg; n=6–7; P<0.05)

Conclusions: Aged mice have enhanced pressor responses to Ang II, in association with augmented inflammation, vasoconstriction and vascular oxidative stress. NLRP3 inflammasome activation does not appear to contribute to Ang II-induced hypertension in aged mice.

HUMAN AMMONIUM EPITHELIAL CELLS REDUCE INFARCT VOLUME, SPLENIC ATROPHY AND LUNG INFLAMMATION FOLLOWING ISCHEMIC STROKE IN MICE.

*Cardiovascular Disease Program, Biomedicine Discovery Institute and Department of Pharmacology Clayton, Victoria, Australia; The Ritchie Centre, Monash Institute of Medical Research, Clayton, Victoria, Australia

Background: The outcome following ischemic stroke is influenced by the extent of brain injury and also the occurrence of bacterial infections within the lung. These infections are promoted by post-stroke immune suppression, a phenomenon characterized by a marked loss of circulating and splenic leukocytes. Stem cells offer great therapeutic potential for stroke patients and may improve stroke outcome via multiple mechanisms. Human ammonium...
epithelial cells (hAECs), which are a placenta-derived stem cell, may exhibit both immune-modulatory and reparative effects. Aim: To examine the effect of hAECs on brain injury and systemic immunosuppression following ischemic stroke.

Methods: Ischemic stroke was induced by 1 h middle cerebral artery occlusion-reperfusion in male C57BL/6J mice aged 7–12 weeks (n=79). Mice were injected with 1x10^6 hAECs or saline (vehicle) i.v. at 0.5 h after reperfusion. Sham operated mice served as controls (n=26). A parallel rod test assessed motor function and coordination after 24 h and 72 h, after which cerebral infarct volume, splenic atrophy, and lung inflammation were assessed. Splenic leukocytes were quantified using flow cytometry. Apoptotic splenocytes were quantified by immunohistochemistry.

Results: Administration of hAECs reduced infarct volume by ~50% at 24 and 72 h after stroke (P<0.005). Reductions in infarct volume by hAECs were associated with improved motor coordination (P<0.005, measured by parallel rod test) and 50% less mortality at 72 h. Treatment with hAECs completely prevented a 3-fold increase in apoptotic cleaved caspase-3-positive splenocytes at 24 h and blunted the stroke-induced reduction in spleen weight at 72 h. Furthermore, treatment with hAECs prevented the loss of splenic leukocytes (monocytes, T cells and particularly B cells) at 72 h. Finally, histological examination indicated markedly less lung inflammation at 72 h in mice treated with hAECs compared to vehicle (P<0.05).

Conclusions: The present data indicate that hAEC treatment improves outcome following ischemic stroke by limiting both brain injury and stroke-induced systemic immunosuppression. Thus, hAECs may be a viable therapy for neuroprotection and for promoting recovery of the immune system following ischemic stroke.

RESISTIN ENHANCES THE CENTRAL EFFECTS OF LEPTIN ON RENAL SYMPATHETIC NERVE ACTIVITY

Habeebullah H, Altschuman Y, Stebbing MJ, Jenkins TA, Bodger E

School of Medical Sciences and Health Innovations Research Institute, RMIT University, Melbourne, Victoria, Australia

Background: Leptin is a well known hormone released from fat tissue and acts centrally to influence metabolic and cardiovascular functions. It increases renal sympathetic nerve activity (RSNA) in response to acute exposure to GTN.

Methods: RSNA, mean arterial pressure (MAP) and heart rate (HR) in anesthetised Sprague-Dawley male rats were recorded before and for 3 hours after intracerebroventricular saline (control, n=5), leptin (7 µg; n=5), resistin (7 µg; n=4) and the combination of both resistin and leptin (n=4). The rate of change of RSNA to a saline stimulus (ΔRSNA) was calculated for each animal at 30 second intervals. The cumulative ΔRSNA was quantified for 5 min after the stimulus.

Results: Leptin alone and alone significantly increased RSNA (74±17% and 50±14%, respectively) (P<0.001 vs. saline). When resistin and leptin were combined there was a significantly greater increase in RSNA (163±23%; P<0.001, compared to either drug alone). Changes in MAP and HR from pre-drug levels were not significantly different between groups. The increase in Fos-positive cell nuclei elicited by resistin was similar to that for leptin. When leptin and resistin were combined, the increase in the number of Fos-positive neurons in the arcuate nucleus and in the lamina terminals was significantly greater than control.

Conclusion: The findings show that leptin and resistin, combined, enhance RSNA as well as Fos production in the arcuate nucleus and lamina terminals. Since leptin makes an important contribution to the elevated RSNA observed in obese/overweight conditions, the increased leptin and resistin levels may mean the contribution of leptin to the elevated RSNA in those conditions is enhanced.

BLOOD PRESSURE, INITIAL ORTHOSTATIC HYPOTENSION, GLYCERYL TRINITRATE AND THE GLU504LYS POLYMORPHISM OF ALDEHYDE DEHYDROGENASE-2

Harper RP, Lamantia A, Ziegas JP, Bourke JE

*Department of Physiology and †Department of Pharmacology & Therapeutics, University of Melbourne, Melbourne, Victoria, Australia; ‡Department of Pharmacology, Monash University, Clayton, Melbourne, Australia

Background: The organic nitrate, glyceryl trinitrate (GTN), is used to treat angina and lower blood pressure through release of nitric oxide (NO). The mitochondrial aldehyde dehydrogenase-2 (ALDH2) can activate GTN. A functional polymorphism Gly504Lys, more common in Asian populations, in the gene encoding ALDH2 is associated with significantly reduced enzyme activity and in some studies diminished responses to GTN.

Aim: To assess, in healthy young adults, systolic blood pressure (SBP) responses to standing before and after GTN and the relationship to ALDH2 genotype.

Methods: 493 medical students (mean age 22 years) consented to continuous measurement of SBP (Finometer Mid) during 2 orthostatic challenges. We recorded the average SBP for 30 seconds after 5 minutes lying, the maximum change in SBP within 30 seconds of standing and the average SBP for 30 after 2 minutes standing. After a control challenge, 390 subjects had a second challenge 5 minutes after taking 300 µg GTN sublingually. The remaining 103 subjects had the second challenge without GTN. All subjects were genotyped for the rs671 SNP responsible for the Gly504Lys polymorphism. Allele G is in the codon for Gly at 504 and allele A is in the codon responsible for the dominant functional change to Lys at 504. SBP was compared between those carrying the G0 genotype and those carrying either the G4 or A4 genotype. SBP comparisons were made by paired analyses between control and GTN challenges and between genotypes with generalized linear model univariate analyses (using SPSS) with adjustments for age, sex and weight.

Results: During the control challenge the mean lying SBP was 117±15.8 SD mmHg, fall- ing to 73±20.1 mmHg on initial standing and stabilizing at 109±16.9 mmHg after 2 min- utes of standing. After GTN, SBP was significantly (all P<0.0001) lower than control lying (114±15.9 mmHg), on standing (80±13.9 mmHg) and after 2 minutes standing (103±16.9 mmHg). In the 103 subjects without GTN there were no significant differences between the SBP measures between the first and second orthostatic challenges. None of the SBP responses after GTN differed between the two ALDH2 genotype groups.

Conclusion: In healthy young adults acute GTN causes significant reductions in the rest- ing, lying and standing SBP and a major exaggeration of the initial orthostatic hypotension within 30 s of standing – a period often associated with syncope. The hypertensive actions of GTN were not associated with genetically determined disparities in ALDH2 activity as defined by the Gly504Lys polymorphism. These findings suggest that factors other than ALDH2 activity explain the blood pressure effects of acute exposure to GTN.

RENAL DENERVATION AMELIORATES THE DECLINE IN KIDNEY FUNCTION IN PATIENTS WITH CHRONIC KIDNEY DISEASE INDEPENDENT OF BLOOD PRESSURE

Herring DR, Manucic P, Duval J, Sata Y, Ester MD, Walton AS, Schlaich MP

*Neuropsychiatric Hypertension & Kidney Disease Laboratory, Baker IDI Heart & Diabetes Institute, Melbourne, Victoria, Australia; †School of Medicine and Pharmacology – Royal Perth Hospital Unit, University of Western Australia, Perth, Western Australia, Australia; ‡Heart Centre Alfred Hospital, Melbourne, Victoria, Australia; §Faculty of Medicine, Nursing and Health Sciences and Department of Physiology, Monash University, Clayton, Melbourne, Victoria, Australia

Background: Renal denervation (RDN) has been shown to reduce blood pressure (BP) and slow the decline of renal function in stage 3 and 4 chronic kidney disease (CKD) patients for up to one year. Whether this effect is maintained beyond the first year and whether the magnitude of BP reduction achieved affects estimated glomerular filtration rate (eGFR) is unknown.

Aim: To investigate the effect of RDN on renal function and BP in CKD patients out to 24 months post procedure.

Methods: We examined renal function in 46 CKD patients with an eGFR <60 ml/min/1.73 m2 on a yearly basis from 60 months before to 3, 6, 12 and 24 months after RDN. 24-hour ABPM was measured before RDN and at follow up.

Results: A significant decline in eGFR was observed from months 60 to 48 (~7.3±8.7), months 48 to 36 (~1.4±8.6), months 36 to 24 (~2.7±6.0), months 24 to 12 (~5.9±7.9 ml/min/1.73 m2) (P<0.001), and from 12 months to baseline prior to RDN by ~3.4±7.9 ml/min/1.73 m2 (P<0.001). RDN was associated with improved eGFR at 3 months (~3.7±6.7 ml/min/1.73 m2) (P<0.05), and 6, 12, and 24 months follow up (~1.8±10.8 ml/min/1.73 m2) and only a small decline of ~1.2±11.3 ml/min/1.73 m2 at 24 months follow up (P=0.05). While there was no significant change in daytime SBP between visits from baseline to 12 months after RDN for the entire cohort, patients with baseline daytime SBP >135 mmHg (~12) experienced a significant reduction in daytime SBP 24 months post procedure (P=0.039). Changes in daytime BP were unrelated to the changes in eGFR at 6 (~P=0.033), 12 (~P=0.01), 24 (~P=0.93) and 24 months (~P=0.42). P=0.17 follow up.

Conclusions: Our findings indicate that in patients with CKD RDN can slow further deterio- ration of renal function irrespective of BP lowering effects. RDN-induced inhibition of sym- pathetic outflow to the renal vascular bed may account for improved eGFR via alterations of intrarenal and glomerular hemodynamics.

CARDIAC REPAIR BY DIFFERENTIATION AND MATURATION OF CARDIOMYOCYTES FROM HUMAN INDUCED PLURIPOTENT STEM CELLS: THE BENEFITS OF SHORT- AND LONG-TERM ELECTRICAL STIMULATION


*O’Brien Institute Department, St Vincent’s Institute of Medical Research, Melbourne, Victoria, Australia; †University of Melbourne, Melbourne; ‡Monash University; †Bionics Institute, Melbourne, Victoria, Australia; ‡Centre for Eye Research Australia, Melbourne, Victoria, Australia; †School of Medicine, Menzies Institute for Medical Research, University of Tasmania, Hobart, Tasmania, Australia. *Equally contributing authors

Background: Regeneration of cardiac tissue raises aspirations for therapeutic restoration of cardiac function after myocardial infarction. The ability of human induced pluripotent stem cells (iPSCs) to differentiate into bona fide cardiomyocytes provides a platform for
disease modelling and drug discovery and testing to improve treatment options. One of the major limitations for the use of cardiomyocytes derived from iPSCs is that they resemble fetal cardiomyocytes and are immature. Considering that the developing heart grows in an electric field, we considered that electrical stimulation (ESI) might affect cardiogenesis of human iPSCs.

Aims: To investigate whether ESI promotes cardiac differentiation and maturation of cardiomyocytes derived from human iPSCs.

Methods and Results: Acute ESI (alternating current, charge-balanced biphasic pulse, 1 ms pulse width, 1 Hz frequency) at 200 μA/mm2 for 5 min increased the percentage of beating embryoid bodies (EBs, 11±2% vs. 4±2% in control, non-stimulated (P<0.05; n=11–15) and gene expression of cardioc-specific contractile muscle markers ACTC1, MYH7, MYH11, and MYL7 (n=7). Beating EBs displayed cyclic changes in intracellular calcium ion and contractile responsiveness to isoprenaline and carbamylcholine. Chronic ESI at 200 μA/mm2 for 7 days significantly increased the percentage of cardiomyocytes with organized sarcomeres (39±8% vs. 23±11%; P<0.05; n=3), aligned in parallel with the electric field (10±1% vs. 6±2%; P<0.05; n=3) and decreased the circularity index (0.69±0.02 vs. 0.74±0.02; P<0.05; n=3) indicating a more rod-like structure. The effects of longer stimulations need to be carefully evaluated. In addition, using a biologic approach, ESI is now being applied locally to cardiomyocytes derived from iPSCs in an in vivo system in rat tissue engineering chambers.

Conclusion: Brief ESI modestly enhanced cardiac differentiation of human iPSCs. Chronic ESI might promote further maturation of cardiomyocytes derived from human iPSCs. Mature cardiomyocytes can recapitulate better the pathophysiological conditions of human heart for more accurate disease modelling and drug testing, as well as providing a substrate for neonatal and adult cardiac regeneration and repair by tissue engineering in the future.

CAN TREATMENT OF YOUNG GENETICALLY HYPERTENSIVE MICE WITH ALLOPREGNANOLONE AMELIORATE THE DEVELOPMENT OF HYPERTENSION?

Johns EMCa,c, Stevenson BRb, Jackson KL, Trang EP, Daven PJ, Evans RG, Head GAa

*Baker IDI Heart & Diabetes Institute, Melbourne, Victoria, Australia; aDepartment of Pharmacology, Monash University, Clayton, Victoria, Australia; cDepartment of Physiology, Monash University, Clayton, Victoria, Australia

Background: In humans, stress-related hypertension is associated with an early predisposition to augmented cardiovascular response to stress. This may result from reduced GABAergic inhibition of specific forebrain nuclei that are responsible for initiating and maintaining the pressor response to stress. Allopregnanolone (alloP) is a positive allosteric modulator of the GABAα receptor. We have shown that this can reduce blood pressure (BP) in adult genetically hypertensive (BPH2J) mice.

Aims: To determine whether treatment of young BPH2J mice with alloP can suppress the development of hypertension.

Methods: Six-week old BPH2J (n=19) and normotensive BPN3J (n=15) mice were treated with 10 nmol/kg/day of alloP or vehicle for 24 weeks. Water intake, systolic blood pressure (SBP) and heart rate (HR) were monitored. At 24 weeks, mice were killed and hearts were removed and weighed.

Results: Six-week-old BPH2J mice treated with alloP had lower levels of neuronal activity in the paraventricular nucleus of the hypothalamus compared to vehicle-treated mice (20% (P<0.02) which ameliorated 36% of their hypertension (P=0.02). The pressure response to dirty cage-switch stress and restraint stress were 20% (P=0.08) and 10% (P=0.03) lower, respectively, in BPH2J mice treated with alloP compared with vehicle. No differences in stress responses or 24-hour BP were observed in BPN3J mice. Mice treated with alloP had lower levels of neuronal activity in the paraventricular nucleus of the hypothalamus and medial amygdala (both (P=0.04) compared to vehicle treated mice. Two weeks after treatment with alloP was terminated, 50% of the hypertensive effect of alloP on 24-hour BP in BPH2J mice was reversed, suggesting that a portion of the cardioprotective effects of alloP persisted after treatment ceased.

Conclusion: Treatment of young mice with alloP effectively attenuated the development of hypertension, but these effects were partly reversed after treatment was withdrawn. This study suggests that targeting GABAα receptors with alloP is a safe and effective long-term treatment for stress-related hypertension.

ROLE OF INSULIN-RESPONSIVE AMINOPETIDASE IN THE REGULATION OF WATER HOMEOSTASIS, ARTERIAL PRESSURE AND RENAL FUNCTION

Kett M, Chai SY, Cai X, Denton KM

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

Background: Insulin-responsive aminopeptidase (IRAP) is present in high concentrations in the kidney, particularly principle cells of the collecting duct. Vasopressin, a substrate of IRAP, via a prostanoid-dependent mechanism in the SHRSP, a widely used animal model of hypertension, regulates arterial pressure. Hypertension is associated with a decrease in renal cortical and medullary tissue oxygen tension (PO2). To investigate whether ESt promotes cardiac differentiation and maturation of cardiomyocytes derived from human iPSCs. Chronic ESI might promote further maturation of cardiomyocytes derived from human iPSCs. Mature cardiomyocytes can recapitulate better the pathophysiological conditions of human heart for more accurate disease modelling and drug testing, as well as providing a substrate for neonatal and adult cardiac regeneration and repair by tissue engineering in the future.

Y CHROMOSOME LINEAGE INFLUENCES IMMUNE-MEDIATED VASCULAR DYSFUNCTION VIA A PROSTANOID-DEPENDENT MECHANISM


*Baker IDI Heart & Diabetes Institute, Melbourne, Victoria, Australia; aDepartment of Pharmacology, Monash University, Clayton, Victoria, Australia; *equal contribution

Background: The hereditary lineage of the Y chromosome is an integral determinant of cardiovascular disease risk in males. Y chromosome lineages account for a 15–20 mmHg difference in arterial pressure where the Y chromosome of some (EC50; SP .WKYGlaY: 7.7±0.1; WKY: 9.7±0.1; P<0.01). Replacing the SHRSP Y chromosome with the normotensive Wistar Kyoto (WKY) Y chromosome (SP.WKY.Y) and vice versa (WKY.SPGlaY). However, the gene(s) and mechanism(s) underlying this are not known. Vascular dysfunction is a hallmark of hypertension associated with augmented vascular T cell infiltration.

Aims: To determine the influence of Y chromosome lineage on immune-mediated vascular dysfunction.

Methods: Standard organ bath methodology, flow cytometry and cytokine bioplex were employed.

Results: We observed impaired endothelium-dependent relaxation in the aorta of the SHRSP compared with the WKY (EC50; acetylcholine: SHRSP: 7.3±0.1 vs. WKY: 7.9±0.1; P<0.01). The SHRSP Y chromosome with the normotensive WKY Y chromosome (EC50; SP.WKY.Y: 7.7±0.1; P<0.01) improved vascular function through a reduction in constrictor prostanoid activity and a reversal of prostacyclin receptor dysfunction. In separate experiments, we showed that aortic T cell infiltration was higher in the SHRSP compared with the WKY (5.1±1.2 vs. 1.6±0.4 x106 cells; P<0.05), and introgression of the alternate Y chromosome reduced infiltration (SP.WKY.Y: 2.0±0.7 x106 cells; P<0.05). Furthermore, T cells isolated from the SHRSP aorta displayed a Th1 cytokine skewing compared with the WKY, as indicated by a higher ratio of interferon gamma to interleukin-4 production that was reduced in SP.WKY.Y aortic T cells. Finally, overnight stimulation of T cells from aortas from all four strains with anti-CD3+ and anti-CD28+ antibodies worsened endothelial function only in SHRSP aortas. Cells from the SHRSP aorta stimulated with anti-CD3+ and anti-CD28+ antibodies were completely attenuated in both WKY and SHRSP aortas, highlighting a close interaction between ROS and cyclooxygenase activity.

Conclusion: Y chromosome lineage influences immune-mediated vascular dysfunction via a prostanoid-dependent mechanism in the SHRSP, a widely used animal model of hypertension.

INTRA-RENAL AND URINARY OXYGENATION DURING NORADRENALINE RESUSCITATION IN CONSCIOUS OVINE SEPTIC ACUTE KIDNEY INJURY

Lankadeva YR, Kosaka J, Evans RG, Bellomo R, May CN

*Forey Institute of Neuroscience and Mental Health, Melbourne, Victoria, Australia; aDepartment of Physiology, Monash University, Clayton, Victoria, Australia; bDepartment of Intensive Care and Department of Medicine, Austin Health, Melbourne, Victoria, Australia

Background: Sepsis is commonly associated with hypotension and acute kidney injury (AKI), leading to high mortality rates in patients. There is increasing evidence that renal tissue hypoxia may play a critical role in the pathogenesis of AKI. Noradrenaline (NA) is the principal vasoconstrictor used to reverse hypotension and maintain renal function in septic patients, but its effect on intra-renal oxygenation is unknown.

Aims: To measure renal cortical and renal medullary tissue oxygen tension (PO2) in conscious sheep during development of septic AKI and the response to resuscitation with NA.

(i) In addition, since the renal vasa recta run close and parallel to the medullary collecting
PROGRAMMING OF OBESITY RELATED HYPERTENSION: AMPLIFICATION OF LEPTIN SIGNALING PATHWAY IN THE VENTRICAL HYPOTHALAMUS

Lim KS, Burke SL, Head GA

aBaker IDI Heart & Diabetes Institute, Melbourne, Victoria, Australia; bDepartment of Pharmacology, Monash University, Melbourne, Victoria, Australia

Background: Obesity during pregnancy is associated with a greater risk of developing hypertension in the offspring. Plasma leptin levels correlate strongly with blood pressure and renal sympathetic nerve activity (RSNA). The ventromedial hypothalamus (VMH) is a key centre of energy homeostasis, hemodynamics and sympathetic tone to the renal vasculature. It is possible that exposure to over-nutrition during development changes the activity of the neurons, amplifying sympathetic output leading to hypertension in the offspring. We assessed the contribution of the leptin and melanocortin (MC) signalling pathway in the VMH of offspring that were born from obese mothers.

Aim: To determine whether maternal obesity plays a role in programming the leptin and melanocortin signaling pathway in the VMH.

Methods: Female New Zealand White rabbits were fed a high fat diet (13%; mHFD) or a control diet (4%; mCD) during pregnancy and lactation. Offspring received a control diet after weaning. All offspring received a VMH cannula and a renal nerve recording electrode. Experiments were conducted in conscious rabbits and mean arterial pressure (MAP), heart rate (HR) and RSNA were measured. Rabbits received increasing doses of α-melanocortin stimulating hormone (α-MSH, 0.3 and 1 nmol), SHU9119 (melanocortin receptor antagonist, 0.02 and 0.04 nmol), leptin receptor antagonist (S and 10 ug) or insulin receptor antagonist (0.01 and 0.05 μl).

Results: mHFD rabbits exhibited higher MAP and RSNA than mCD rabbits (P<0.05). α-MSH injection into the VMH increased MAP (+6%), HR (+12%) and RSNA (+80%) over baseline, SHU9119 reduced MAP (~7%) in mHFD rabbits. Leptin receptor antagonist normalized hypertension in mHFD rabbits (P<0.05). By contrast, no changes were observed following insulin receptor antagonist injections into the VMH, mCD did not respond to any drug injections into the VMH.

Conclusion: Exposure to over-nutrition during development alters the leptin and MC signaling pathway in the VMH of the offspring.

INHIBITION OF INTERLEUKIN-1 β SIGNALING WITH ANAKINRA REDUCES BLOOD PRESSURE BUT NOT RENAL INFLAMMATION AND DAMAGE IN MICE WITH ONE-KIDNEY/DOCA/SALT-INDUCED HYPERTENSION

Ling YH*, Krishnan SM, Chan CT, Diep H, Samuel CS, Hewitson TD, Mansell A, Lambert GW*

aDepartment of Pharmacology, Monash University, Clayton, Victoria, Australia; bDepartment of Nephrology, Royal Melbourne Hospital, Parkville, Victoria, Australia; cHudson Institute of Medical Research, Clayton, Victoria, Australia

Background: Hypertension is a chronic inflammatory disease, with the kidneys being a major site of inflammation. We have shown that inflammatory activation and interleukin-1 (IL-1)β production are crucial for renal inflammation and elevated blood pressure (BP) in experimental models of hypertension in mice.

Aim: To determine whether a clinically utilized IL-1 receptor antagonist (anakinra) can reduce renal inflammation and damage, with or without BP lowering in mice with established one-kidney (1K)/DOCA/salt-induced hypertension.

Methods: Hypertension was induced in male C57BL/6J mice by unconstrained, treatment with deoxycorticosterone (DOCA; 2.4 mg/d, s.c.) and replacement of drinking water with saline (1K/DOCA/salt). Control mice were uninephrectomized and received a placebo pellet. Treatment with anakinra was initiated at 8 weeks of age with anakinra (4 mg/kg, s.c.) or vehicle (0.9% saline, s.c.) for 32 weeks. The mice were killed at 32 weeks, and renal collagen content/fibrosis was assessed by measuring hydroxyproline content. Renal damage was assessed by calculating kidney/body weight ratios and by measuring glomerular surface area in kidney sections.

Results: By 10 days post-surgery, 1K/DOCA/salt-treated mice displayed markedly elevated systolic BP (148.3±2.4 mmHg) compared to control mice (121.7±2.7 mmHg; n=18; P<0.0001). The intervention with anakinra reduced BP in 1K/DOCA/salt-treated mice by up to 20 mmHg (n=16; P<0.05), yet had no effect on this parameter in control mice. Real-time PCR showed that anakinra reduced renal expression of some inflammatory markers in 1K/DOCA/salt-treated mice (e.g., CCL5 and CCL2; n=7–8; P<0.05). Anakinra also failed to reduce collagen content in the kidneys of 1K/DOCA/salt-treated mice and actually worsened renal damage as demonstrated by further increases in kidney weight (n=6; P<0.001) and size of glomeruli (n=8–9; P<0.001).

Conclusion: Despite its anti-hypertensive actions in mice with established 1K/DOCA/salt hypertension, anakinra had minimal effects on renal inflammation and leukocyte infiltration and may exacerbate renal damage. Future studies will assess whether the anti-hypertensive actions of anakinra are protected by reactive actions in other BP regulating organs such as the arteries and brain.

TRANSCARDIAC GRADIENT OF CARDIO-mICRONAS IN THE FAILING HEART

Marques FZ, Vizi D, Khrammy O, Mariani JA*, Kaye DM*

aHeart Failure Research Group, Baker IDI Heart and Diabetes Research Institute, Melbourne, Victoria, Australia; bThe Heart Centre, Alfred Hospital, Melbourne, Victoria, Australia

Background: Circulating microRNAs have been associated with heart failure and could potentially be used as biomarkers for diagnosis and disease pathogenesis. Whether microRNAs accurately reflect cardiac turnover rather than systemic disturbances is unclear.

Aim: To determine the transcardiac gradient of 84 microRNAs involved in cardiovascular development and disease in failing and non-failing hearts.

Methods: Eight healthy volunteers and 9 patients with congestive heart failure were included in this study. Arterial and coronary sinus blood samples were collected simultaneously and microRNAs were extracted from plasma. The expression of microRNAs was analysed using real-time semi-quantitative PCR by the miScript miRNA PCR Array Human Cardiovascular Disease. The transcardiac gradient was calculated by subtracting microRNA expression levels in arterial blood from microRNA levels in coronary sinus samples. A P value of <0.05 was considered significant.

Results: In coronary sinus samples, the microRNAs miR-18-5p, miR-27a-3p, miR-27b-3p, miR-29b-3p, miR-29c-3p, miR-30e-5p, miR-92a-3p, miR-125b-5p, miR-140-5p, miR-195-5p, miR-424-5p and miR-451a were significantly down-regulated, and let-7a-5p, let-7c-5p, let-7e-5p, miR-23b-3p, miR-107, miR-155-5p, miR-181a-5p, miR-181b-5p and miR-320a were up-regulated in heart failure. Left ventricular filling pressure was negatively correlated with miR-195, miR-16, miR-23b-3p, miR-29c-3p, miR-451a and miR-92a-3p, and all had receiver operating characteristic analysis between 0.806 and 0.875. miR-140-5p was the only microRNA released from the healthy heart, while the failing heart released let-7b-5p, let-7c-5p, let-7e-5p, miR-122-5p and miR-21-5p, and absorbed miR-23b-3p, miR-107, miR-155-5p, miR-181a-5p, miR-181b-5p and miR-320a.

Conclusion: The transcardiac gradient of cardio-microRNAs in failing hearts was determined. The results support the use of some microRNAs as potential biomarkers and therapeutical targets. MicroRNAs identified in the present study are likely to have a role in the development and disease in healthy and failing hearts.

A POLYMORPHISM IN A microRNA-BINDING SITE IN THE MESSANGER RNA FOR THE NORDRANELINE TRANSPORTER MAY INCREASE RISK OF CARDIOVASCULAR DISEASE DEVELOPMENT

Marques FZ, Eikelis N, Lambert EA*, Schlais MP**, Esler MD**, Barton DA**, Lambert GW*

aHeart Failure Research Group; bHuman Neurotransmitters and Neurovascular Hypertension & Kidney Disease Laboratories, Baker IDI Heart and Diabetes Research Institute, Melbourne, Victoria, Australia; cDepartment of Physiology and dDepartment of Medicine, Monash University, Melbourne, Victoria, Australia

Background: Nordraneline released from sympathetic nerves is removed from the synapse via the action of the noradrenaline transporter (NET). NET impairment is evident in several clinically important conditions, including essential hypertension, major depressive disorder, panic disorder and the postural orthostatic tachycardia syndrome. Only in rare instances, however, do coding single nucleotide polymorphisms (SNPs) seem to account for a defect in NET.

Aim: To determine whether rs7194256 (G/T) in the 3’ untranslated region (UTR) of the mRNA of the gene NET is associated with diseases associated with NET dysfunction, and to elucidate the mechanism involved.
Methods: We genotyped by real-time semi-quantitative PCR (qPCR) the rs7194256 SNP in a cohort of 122 patients (including 64 hypertensives) and 55 healthy controls, all of European-descent, and validated the results in a larger cohort of 258 cases (124 hypertensive) and 238 controls. Bioinformatic analyses were then used to identify microRNAs that could bind to the RNA sequence in which the T allele was present. The effect of the T allele on expression of a luciferase reporter gene was then examined.

Results: Cases had significantly higher prevalence of the T allele, arterial noradrenaline, depression and anxiety scores, clinical and ambulatory systolic and diastolic blood pressures, and larger left ventricular mass index (all P<0.05). Carriers of the T allele also had higher arterial noradrenaline (P=0.002) and 3,4-dihydroxyphenylglycol (the intraneuronal metabolite of noradrenaline; P=0.016). Bioinformatic analyses showed that the presence of the T allele created a binding site for the microRNA mir-19a-3p. Luciferase reporter gene assays validated the ability of this microRNA to bind preferentially to the sequence containing the T allele (P<0.0001).

Conclusion: The T allele of the SNP rs7194256 when present in the 3’UTR of the NET mRNA is associated with diseases associated with NET dysfunction, including hypertension. This might be explained by the presence of a binding site for the microRNA mir-19a-3p in NET mRNA. A defect in NET function may potentiate the sympathetic nervous system, predisposing individuals to increased risk of cardiovascular disease development.

PSAMLOTOX ANOXIS NEUROPROTECTION IN A CONSCIOUS MODEL OF STROKE IN HYPERTENSION RATS VIA SELECTIVE INHIBITION OF ACID-SENSING ION CHANNEL 1A
McCarthy GA, Rash LD, Chassagnon RP, King GP, Widdop RE
*Department of Pharmacology, Monash University, Clayton, Melbourne, Victoria, Australia; ^Institute for Molecular Bioscience, The University of Queensland, Brisbane, Queensland, Australia

Background: Acid-sensing ion channel 1a (ASIC1a) is the primary acid sensor in mammalian brain and plays a major role in neuronal injury following cerebral ischaemia. Evidence that inhibition of ASIC1a might be neuroprotective following stroke was previously obtained using psalmotrin (PcTx1) from the venom of the tarantula, Psalmopoeus cambridgei. Since the ASIC1a-selective blocker PcTx1 is present at only 0.4% abundance in this venom, we wondered whether the observed neuroprotective effects were due to PcTx1 blockade of ASIC1a or inhibition of other ion channels and receptors by the hundreds of peptides and small molecules present in tarantula venom.

Aim: To examine whether pure PcTx1 is neuroprotective in a conscious model of stroke via direct inhibition of ASIC1a.

Methods: A focal ischaemia model of stroke was induced in conscious spontaneously hypertensive rats (SHR) by administering endothelin-1 to the middle cerebral artery (MCA) via a surgically implanted cannula. Two hours later SHR were treated with a single intra-cerebroventricular dose of PcTx1 (1 ng/kg, n=9), an ASIC1a-inactive mutant of PcTx1 (1 ng/kg; n=7), or saline (n=10). Motor coordination was measured at 1 and 3 days after stroke and post mortem analyses of cortical and striatal infarct volumes, neuronal survival and apoptosis were performed 72 hours post MCA occlusion.

Results: PcTx1 markedly reduced cortical infarct volume from 108±22 mm² in vehicle-treated SHR to 32±10 mm² in PcTx1-treated SHR (P<0.05) and reduced striatal infarct volume from 39±6.5 mm² (vehicle) to 24±2.8 mm² (PcTx1-treated). Motor coordination was measured at 1 and 3 days after stroke and post mortem analyses of cortical and striatal infarct volumes, neuronal survival and apoptosis were performed 72 hours post MCA occlusion.

Conclusion: The present study is the first to demonstrate that selective pharmacological inhibition of ASIC1a is neuroprotective in conscious SHR, thus validating inhibition of ASIC1a as a potential treatment for stroke.

ASSOCIATION ANALYSIS OF FOXO3 LONGEVITY VARIANTS WITH BLOOD PRESSURE AND ESSENTIAL HYPERTENSION
Morris B1, Chen R1, Donlon TA1, Evans DS2, Tranah GJ3, Parimi N1, Ehret GB4, Morris BJ1-3, Chen R1, Donlon TA1, Evans DS2, Tranah GJ3, Parimi N1, Ehret GB4
1Department of Prosthodontics, Gerodontology and Oral Rehabilitation, Osaka University Graduate School of Dentistry, Suita, Japan; 2Tokyo Metropolitan Geriatric Hospital and Institute of Gerontology, Tokyo, Japan; 3Keio University, School of Medicine Tokyo, Japan; 4Department of Clinical Thanatology and Geriatric Behavioral Science, Osaka University Graduate School of Human Sciences, Suita, Japan

Background: The minor alleles of three FOXO3 single nucleotide polymorphisms (SNPs) −rs2802292, rs2253310 and rs2802288 − have been consistently associated with human longevity in all studies worldwide.

Aim: To test these SNPs for association with blood pressure (BP) and essential hypertension (EHT).

Methods: In a primary study involving Americans of Japanese ancestry drawn from the Family Blood Pressure Program II we genotyped 411 female and 432 male subjects aged 40–79 years and tested for statistical association by contingency table analysis and generalized linear models that included logistic regression adjusting for sibling correlation in the data set. Replication of SNP rs2802292 with EHT was attempted in Japanese SONIC study subjects and of each SNP in a meta-analysis of genome-wide association studies of BP in individuals of European ancestry.

Results: In Americans of Japanese ancestry, women homoyzgous for the longevity-associated allele of each FOXO3 SNP had 6 mmHg lower systolic BP and 3 mmHg lower diastolic BP compared with major allele homozygotes (Bonferroni corrected P<0.05 and >0.05, respectively). Frequencies of minor allele homozygotes were 3.3–3.9% in women with EHT compared with 9.5–9.6% in normotensive women (P=0.03–0.04, haplotype analysis P=0.0002). No association with BP or EHT was evident in males. An association with EHT was seen for the minor allele of rs2802292 in the Japanese SONIC cohort (P=0.03), while in European subjects the minor allele of each SNP was associated with higher systolic and diastolic BP.


FACEBOOK ADVERTISING IS SUCCESSFUL FOR PARTICIPANT RECRUITMENT INTO A NATIONAL BLOOD PRESSURE CLINICAL TRIAL
Nash E, Sharmen JE
Menzies Institute for Medical Research, University of Tasmania, Hobart, Tasmania, Australia

Background: Recruiting samples of sufficient size into clinical trials is challenging. Conventional recruitment methods, such as popular press advertisements, are often ineffective. Facebook advertisement has been used successfully to recruit younger participants, but the effectiveness of this social media tool for recruitment into blood pressure clinical trials of older people is unknown.

Aim: To evaluate the use of Facebook advertisement to recruit participants into a national, randomized controlled clinical trial related to blood pressure.

Methods: Conventional advertisements (newspaper, radio and posters in doctors clinics) were employed for the first 20 months of a clinical trial (LOW9BP) conducted in the Australian state capital cities of Hobart, Brisbane and Canberra. With dwindling participant recruitment, a Facebook advertising campaign, targeting adults up to 69 years of age who were currently taking blood pressure medication was employed in each city. Campaigns were broadcast intermittently over a 4-month period, with recruitment results compared to those using conventional methods in the previous 20 months.

Results: Overall, there was a significant increase in participants recruited using Facebook advertisement compared with conventional methods (from 3.8/month to 6.2/month; P<0.01). Compared with the period 4 months prior to Facebook advertisement, there was improved participant recruitment in Hobart (from 4.0/month to 9.3/month; P<0.05) and Canberra (1.8/month to 6.5/month; P<0.05), but there was no change to recruitment in Brisbane (2.4/month to 2.8/month; P=0.89). Despite a greater population reach in Brisbane (n=31,929) compared with Canberra (n=71,343) and Hobart (n=52,647), the number of clicks on to the advertisement in Brisbane was equal to other sites (n=2575, n=2521, n=2991, respectively).

Conclusion: Although effectiveness may be location-dependent, Facebook advertisement was highly successful in increasing participant recruitment into a clinical trial blood pressure trial in older people.

ACUTE BLOOD PRESSURE RESPONSE TO ANTIHYPERTENSIVES DURING ANESTHESIA IN AN EXPERIMENTAL MODEL OF PREECLAMPSIA
Pears S1, Sunderland N, Dennis A1,2, Lim S4, Chau K4,5, Aggarwal S1, Heffernan S1, Downey R1, Ogle R1, Iliopoulos J2, Hennessy K1,3, Makris A1,2
1Royal Prince Alfred Hospital, Sydney, New South Wales, Australia; 2University of Sydney, Sydney, New South Wales, Australia; 3The Royal Women’s Hospital, Melbourne, Victoria, Australia; 4University of Melbourne, Melbourne, Victoria, Australia; 5Campbelltown Hospital, Sydney, New South Wales, Australia; 6Heart Research Institute, Sydney, New South Wales, Australia; 7Liverpool Hospital, Sydney, New South Wales, Australia; 8Western Sydney University, Sydney, New South Wales, Australia

Background: Increased blood pressure (BP), especially sudden, unexpected and severe rises in women with preeclampsia considerably increases the risk of peripartum complications. These risks are even more acute in the context of general anesthesia.
Aim: To determine the BP effect of commonly used antihypertensives during anesthesia in an experimental model of preeclampsia.

Methods: Blood pressure was measured during anesthesia in 6 pregnant baboons (Papio hamadryas) with experimental preeclampsia (PE). Animals were given antihypertensives commonly used to manage preeclampsia (labetalol, methyldopa and hydralazine) at equivalent doses equivalent to mild starting dose rates commonly used in women with preeclampsia. Results: When anesthetized with ketamine (the most commonly used anesthetic agent in EPE), systolic BP increased significantly by 4.9 mmHg when animals were on labetalol (n=3; P<0.05), decreased by 0.5 mmHg when animals were on methyldopa (n=3; NS) and decreased by 4.9 mmHg when animals were on hydralazine (n=2; NS) as compared to systolic BP under ketamine anesthesia prior to receiving any medication. With propofol anesthesia (commonly used when anesthesia is required in women with preeclampsia), systolic BP decreased by 1.4 mmHg when animals were on labetalol (n=3; NS), decreased by 7.2 mmHg when animals were on methyldopa (n=2; NS) and increased by 2.8 mmHg when animals were on hydralazine (n=3; NS) as compared to systolic BP under propofol anesthesia prior to receiving any medication.

Conclusion: The present results show that the two anesthetic agents studied the greatest reduction in BP in animals achieved with hydralazine during ketamine anesthesia and methyldopa during propofol anesthesia. It is likely that antihypertensive treatment affects BP during anesthesia, and that the interaction between antihypertensive and anesthetic agent is of clinical importance in managing sudden, unexpected and severe rises in BP in women with preeclampsia.

EFFICACY OF RENAL DENERVATION IN A RABBIT MODEL OF CHRONIC KIDNEY DISEASE

Saha V, Burke SL, Schlaich MP, Head GA

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

Background: Chronic kidney disease (CKD) is an increasing disease burden affecting nearly 1 in 20 Australians. CKD is associated with activation of the sympathetic nervous system and elevated blood pressure (BP). Renal denervation is a commonly used method of determining the role of the renal nerves in the maintenance of blood pressure (BP) in a number of animal models. In hypertensive patients, the efficacy of renal ablation increases between 1 and 3 months after the procedure. We have developed a CKD model in rabbits which showed hypertension and elevated renal sympathetic nerve activity (RSNA). Whether the elevated blood pressure is dependent on intact renal afferent or efferent nerves remains unclear.

Aim: To examine cardiovascular changes after renal denervation over a 4 week period in this model.

Methods: CKD was induced by lesioning 5/8th of the glomerular layer of the renal cortex in one kidney and removing the contralateral kidney. We examined the role of the renal nerves by denervating the kidneys after 2 weeks of CKD. Blood parameters, BP, HR and RSNA were examined in the 4 weeks following denervation (CKD+RNX; n=4) or sham denervation (CKD+sham; n=4).

Results: After induction of CKD, BP increased by 16% from baseline BP of 67±1 mmHg (n=8; P<0.001) but heart rate decreased by 4% (P<0.05). BP in the CKD+sham group continued to rise and at week 4 was 9% higher than pre-denervation BP of 80±1 mmHg. By contrast, BP in the CKD+RNX group did not change after denervation and over 4 weeks there was a marked difference between the groups (P<0.001). In the CKD+RNX rabbits, heart rate was 7% lower after denervation, which was not observed in the CKD+sham group (P=0.03). RSNA, measured over 4 weeks following renal denervation, was also lower in the CKD+RNX (82±8.6 µnu) vs. the CKD+sham group (102±2.8 µnu; P<0.001). Plasma creatinine and urea were 60% and 99% greater, respectively, than baseline at 1–2 weeks after inducing CKD (n=8; P<0.001). There was a fall in both parameters over the next 4 weeks and was similar in CKD+RNX and CKD+sham rabbits.

Conclusion: Our results suggest that the renal nerves make a major contribution to the hypertension associated with CKD in a rabbit model and that renal denervation may be a suitable treatment for CKD.

POTENTIAL ROLE OF THE SYMPATHETIC NERVOUS SYSTEM IN REGULATION OF THE SODIUM GLUCOSE CO-TRANSPORTER 2

Schlaich MP, Elliott RH, Rudnicken C, Matthews VB

Dobney Hypertension Centre, School of Medicine and Pharmacology – Royal Perth Hospital Unit, University of Western Australia, Perth, Western Australia, Australia

Background: Sympathetic nervous system activation is a common feature in various metabolic disorders such as obesity, metabolic syndrome, and type 2 diabetes. The sodium glucose co-transporter 2 (SGLT-2) mediates renal glucose re-absorption. SGLT-2 expression in HK2 cells in response to treatment with NE was determined by immunocytochemistry at 24, 48 and 72 h post-treatment.

Results: A marked increase in SGLT-2 expression was demonstrated after treatment with NE. Furthermore, 0.1 and 0.01 µM of NE resulted in significantly increased IL-6 release from HK2 cells compared to the 0 µM negative control.

Conclusion: Our in vitro study provides the first evidence to suggest that SGLT-2 is up-regulated by NE and indicates the potential role for sympathetic regulation of SGLT-2-mediated renal glucose re-absorption.

BARORECEPTOR SENSITIVITY IS RELATED TO TREATMENT DELAY IN AMBULANT MULTIPLE SCLEROSIS SUBJECTS – A NON-INVASIVE AUTONOMIC FUNCTION ANALYSIS

Shirani E, Barin E, Lee YC, Ng K, Buttlin M, Avolio A, Parratt J

1Macquarie University Hospital Clinic, Sydney, New South Wales, Australia; 2Department of Neurology, Royal North Shore Hospital, University of Sydney, Sydney, New South Wales, Australia

Background: Studies show that autonomic dysfunction relates to progression and extent of disease in multiple sclerosis (MS). Beat-to-beat baroreceptor sensitivity (BRS) measured by the sequence technique (ST) provides a near-instantaneous measure of intrinsic baroreceptor tone. The baroreflex mediates arterial blood pressure responses driven by sympathetic autonomic tone.

Aims: To assess differences in short-term beat-to-beat non-invasive BRS, as well as heart rate variability (HRV), in MS subjects treated with immunomodulatory therapy early versus later in the course of their disease.

Methods: Patients (n=39; age 49±13 SD years; 13 male) were studied using a finger cuff pressure device (Edwards Nexfin®). Recordings of 5 minutes were obtained and intrinsic BRS curves were derived by ST, then correlated with vasomotor, HRV and clinical characteristics, including disability. Subjects treated early (ET < 2 years) or later (NET > 2 years) were categorized into low BRS values (below the median value of 9.98) or high BRS those above the median. Electrocardiograms, time-based HRV and spectral power analysis (PSA) of HRV at very low frequencies (VLF < 0.04 Hz), low frequencies (LF 0.04–0.15 Hz) and high frequencies (HF 0.15–0.4 Hz) were also measured. The Mann-Whitney U test was used to compare inter-group differences, and χ² was used for comparison of proportions, with significance set at P<0.05.

Results: Average duration of diagnosis of MS was 14±2 years. ET vs. NET patients were younger (40±11 vs. 53±12 years; P<0.01) and had relatively higher BRS (9.1±1 in ET vs. 10/18 in NET; P<0.01). The mean BRS (mMg/s) was 13.8±6.6 in ET vs. 10.3±5.2 in NET (P<0.07; not significant). In high BRS subjects PSA HF power (ms²) was 2621±2496 in ET vs. 3430±625 in NET (P<0.01), with no differences at other frequencies. In low BRS subjects HF power was 590±73 in ET vs. 456±213 in NET (P<0.01). Mean square success score (MSSS) values were 2.3±1.5 vs. 2.5±1.9 (P<0.01) in ET vs. NET, respectively.

Conclusions: Intrinsic BRS measures studied were higher in MS subjects treated early in the course of their disease. This was not explained by disability status scores accounting for time (MSSS) and raises the possibility that earlier treatment influences autonomic function in MS. Given that altered cardiac autonomic tone is an adverse prognostic factor in cardiovascular disease and cardiovascular mortality is overrepresented in MS, the impact of immunomodulatory medication on autonomic preservation warrants further study.

RADIO-FREQUENCY CATHETER-BASED RENAL DENERVATION IN HYPERTENSIVE SHEEP WITH CHRONIC KIDNEY DISEASE IMPAIRS RESPONSES TO HEMORRHAGE

Singh RP, Booth LC, May CN, Head GA, Moritz KM, Schlaich MP, Denton KM

1Department of Physiology, Monash University, Melbourne, Victoria, Australia; 2Florey Institute of Neuroscience and Mental Health, Parkville, Melbourne, Victoria, Australia; 3Baker ID Heart & Diabetes Institute, Melbourne, Victoria, Australia; 4The University of Queensland, St Lucia, Brisbane, Queensland, Australia; 5The University of Western Australia, Perth, Western Australia, Australia

Background: Renal sympathetic nerves modulate kidney function and blood pressure. Trials using catheter-based renal denervation (cDNX) in hypertensive patients yielded results both in support of and against its efficacy in lowering blood pressure (BP). A critical question is whether cDNX has adverse consequences in situations of clinical challenge, such as hemorrhage in denervated patients.

Aims: To examine the consequences of cDNX (i) on basal BP and renal function and (ii) in response to reflex activation of sympathetic nerve activity (SNA) triggered by hemorrhage in hypertensive sheep with chronic kidney disease (CKD).

Methods: Sheep with established hypertension and renal dysfunction (CKD group) with an appropriate control group were used. At 10 months of age, some animals underwent cDNX (CKD-cDNX; control-cDNX) while the remaining underwent sham procedure (CKD-intact; control-intact).

Results: Sheep with hypertension and CKD that underwent cDNX had similar BP to control sheep. Sheep that underwent cDNX had significantly greater urinary excretion of sodium (control-cDNX vs. control-intact; P=0.01; CKD-cDNX vs. CKD-intact; P=0.04) compared to their intact counterparts. In response to hemorrhage, BP fell in all groups but the greatest decrease occurred in CKD-cDNX. In control-intact sheep this fall in BP gradually recovered, associated with 0.01, 0.01, 0.01, 0.01, 0.01, 0.01, 0.01, 0.01, 0.01 and 0.01 in PRA reflecting an increase in reflex SNA. In contrast, in control-cDNX and CKD-cDNX groups, PRA did not increase and BP did not recover, reflecting an absence of increase in reflex SNA.
AGE-DEPENDENT BLOOD PRESSURE DIFFERENCES OVER CONSECUTIVE MEASUREMENTS: IMPLICATIONS FOR HYPERTENSION DIAGNOSIS AND GUIDELINES

Veloudi Pa, Blizzard La, Veloudai KSa, Schultz MGa, Sharman JEa

1Menzies Institute for Medical Research, University of Tasmania, Hobart, Tasmania, Australia; 2Department of Medicine, School of Clinical Sciences at Monash Health, Monash Medical Centre, Melbourne, Victoria, Australia

Background: There is anecdotal belief that clinic blood pressure (BP) decreases over consecutive measurements. This has led to some international guidelines to recommend that the first BP reading should be discarded, or that only one reading should be used if systolic BP (SBP) is <140 mmHg. However, the magnitude and direction of the SBP difference between consecutive measurements is not clear, and the effect of age and BP level on this difference is unknown.

Aims: To investigate (i) the interaction between SBP level and age on the differences in SBP observed over consecutive measurements, and (ii) the consequent effect on hypertension diagnosis.

Methods: Duplicate BP (or triplicate if large BP differences) was recorded by oscilometry among 20,318 participants (46 years [95% CI 46, 47]; males 50%) from the 2011–2013 Australian Health Survey. Primary outcome was the absolute difference between the first two SBP readings. Reclassification of BP category was defined as the change of a participant’s BP status either from hypertension at SBP1 to normal SBP, or from normal SBP at SBP1 to hypertension based on: (1) the average of SBP1 and SBP2, (2) the average of SBP1, SBP2 and SBP3 and (3) the average of the SBP2 and SBP3, discarding SBP1.

Results: SBP decreased between the first two measurements in 56%, but increased in 37% and did not change in 7% of the population. There was a strong, age-dependent, J-curved relationship between the difference in SBP from reading 1 to reading 2 and SBP level (P<0.001), with the smallest difference between readings corresponding to controlled SBP (<140 mmHg). The age-dependent difference in SBP resulted in significant diagnostic reclassification compared to an approach of discarding the first reading, with 63% and 35% reclassified from hypertension to normal BP, and 4% and 13% reclassified from normal to hypertension among those aged <50 years and ≥50 years, respectively.

Conclusions: The assumption that SBP decreases over consecutive measurements is false and significant age- and BP-dependent reclassification of hypertension diagnosis exists across different diagnostic protocols. These findings highlight the need for change to some international hypertension guidelines.

THE EFFECT OF MOXONIDINE ON ANGIOTENSIN II-INDUCED ABDOMINAL AORTIC ANEURYSM IN MICE

Wang Y, Dinh TN, Parker K, Arahahi A
School of Applied and Biomedical Sciences, Federation University Australia, Ballarat, Victoria, Australia

Background: Abdominal aortic aneurysm (AAA) affects ~5% of men aged >55 years and causes significant morbidity and mortality. Hypertension is regarded as a risk factor for the development and progression of AAA in humans. However, the study of AAA pathogenesis in humans is limited and there is no pharmaceutical treatment for patients with AAA. The use of appropriate animal models would have an important role in broadening an understanding of the pathogenesis of human AAA and in developing new therapies for AAA.

Aim: To assess the effect of moxonidine, a blood pressure (BP)-lowering drug, on the development of AAA in mice induced by angiotensin (Ang) II infusion.

Methods: Four groups (n=10 per group) of apolipoprotein E-deficient male mice were used. These animals received moxonidine in drinking water at concentrations of 0 mg/ml (control), 3.6 mg/ml (low dose), 15.4 mg/ml (medium dose) and 69.2 mg/ml (high dose) throughout the experiment. Three days after the initiation of the moxonidine treatment, AAA was induced by subcutaneous infusion of Ang II for 28 days. BP was measured by the tail cuff method at baseline, 2 weeks and 4 weeks after AAA induction by Ang II infusion.

Results: The aortic diameter was assessed by morphometric analysis. The mortality rate due to aortic rupture was analysed by constructing survival curves.

Results: Ang II infusion significantly increased BP in mice. Moxonidine treatment, at every dose studied, significantly reduced BP. However, moxonidine treatment did not alter the maximum diameter of the aortic arch, thoracic aorta, suprarenal abdominal aorta and infrarenal abdominal aorta. In addition, moxonidine did not alter mortality rate.

Conclusion: The present study found that moxonidine does not affect the development of AAA induced by Ang II infusion in mice.
locally activated, by an antigen within the vessel wall, indicators of antigen presentation are slower T cell velocities, greater interaction time and a greater proportion of T cells interacting with antigen presenting cells (APCs).

**Aims:** To (i) identify whether cognate antigens are presented to T cells within the vessel wall of hypertensive mice and (ii) elucidate their direct effect on vascular and endothelial function.

**Methods:** Splenic T cells were isolated from normotensive vehicle-treated (nT cells) and hypertensive angiotensin (Ang) II-infused (10 mg/kg/day for 14 days; nT cells) C57BL/6 mice. Following anti-CD3/CD28 stimulation for 48 hours, cells were fluorescedally labeled and co-incubated simultaneously for 16 hours with explanted aorta from normotensive or hypertensive Cd11c-YFP mice, in which APCs are fluorescently labeled. Vascular function was studied using isolated aortic rings, whereby the local vascular T cells were activated by incubating aorta with anti-CD3/CD28 antibodies for 16 hours prior to vascular reactivity studies.

**Results:** In Cd11c-YFP mouse aorta alone, we detected a ~2-fold increase in CCR5 ligand (CCL3, CCL4 and CCL5) secretion from hypertensive mouse aorta compared to vehicle-treated mouse aorta (P<0.05; n=4). Using 2-photon microscopy, we observed an ~2-fold higher number of T cells compared to T cells within Ang II-infused mouse aorta (390±113 vs. 198±49). Importantly, time-lapse imaging of hypertensive mouse aorta showed that T cells exhibited significantly slower velocity (T cells 2.6 µm/min vs. 4.4 µm/min for nT cells; P<0.01; n=8–11), longer duration of interaction (T cells 36.0±5.2 min vs. 25.7±5.1 min for nT cells; P<0.01; n=8–11) and a greater proportion of interactions with APCs (T cells 10.7±2.3% vs. 1.5±0.7% for nT cells; P<0.01; n=8–11). Direct activation of local vascular T cells exacerbated Ang II-induced endothelial dysfunction (67±5.2% vs. 54.5±3.7% maximal relaxation for Ang II alone; P<0.05; n=7–14).

**Conclusion:** The present data provide the first evidence that vascular infiltrating T cells are presented with cognate antigens by APCs within the vessel wall during hypertension and that direct activation of these T cell infiltrates further impairs endothelial function, thereby promoting the development of hypertension.

**HYPERTENSION-INDUCED SYMPATHETIC ACTIVITY: ROLE IN STEM CELL MOBILIZATION, MONOCYTOSIS AND ATHEROSCLEROSIS**

Whillas AT³,b, Al-Sharea Aa,b, Kraikman M, Jefferis AMb, Shihata Wa,b, Sampson AKa,b, Head GP, Andrews KL², Murphy AJb, Chin-Dusting JPFa,b

**Aims:** To (i) identify whether cognate antigens are presented to T cells within the vessel wall of hypertensive mice and (ii) elucidate their direct effect on vascular and endothelial function.

**Methods:** We found that this series of events can be blocked by SNS antagonism.

**Results:** In C57BL/6 mice, we detected a ~2-fold increase in CCR5 ligand (CCL3, CCL4 and CCL5) secretion from hypertensive mouse aorta compared to vehicle-treated mouse aorta (P<0.05; n=4). Using 2-photon microscopy, we observed an ~2-fold higher number of T cells compared to T cells within Ang II-infused mouse aorta (390±113 vs. 198±49). Importantly, time-lapse imaging of hypertensive mouse aorta showed that T cells exhibited significantly slower velocity (T cells 2.6 µm/min vs. 4.4 µm/min for nT cells; P<0.01; n=8–11), longer duration of interaction (T cells 36.0±5.2 min vs. 25.7±5.1 min for nT cells; P<0.01; n=8–11) and a greater proportion of interactions with APCs (T cells 10.7±2.3% vs. 1.5±0.7% for nT cells; P<0.01; n=8–11). Direct activation of local vascular T cells exacerbated Ang II-induced endothelial dysfunction (67±5.2% vs. 54.5±3.7% maximal relaxation for Ang II alone; P<0.05; n=7–14).

**Conclusion:** The present data provide the first evidence that vascular infiltrating T cells are presented with cognate antigens by APCs within the vessel wall during hypertension and that direct activation of these T cell infiltrates further impairs endothelial function, thereby promoting the development of hypertension.

**LOW DOSE DIETARY NITRATE IMPROVES ENDOTHELIAL DYSFUNCTION IN THE APOE–/– MOUSE**

Bakker JP, Bondono NP, Croft KD, Hodgson JM, Kemp-Harper B, Gaspar T, Ward NC²

**Aims:** To determine if dietary nitrate could protect against endothelial dysfunction and lesion formation in the ApoE–/– mouse fed a high fat diet (HFD).

**Methods:** ApoE–/– were randomized to receive either (i) high nitrate (10 mmol/kg/day; n=12), (ii) moderate nitrate (1 mmol/kg/day; n=8), or (iii) low nitrate (0.1 mmol/kg/day; n=8) in drinking water for 10 weeks. A group of ApoE–/– receiving sodium chloride in drinking water (n=10) served as control, while a group of C57BL/6 mice (n=6) receiving tap water served as a healthy reference group. All mice were fed a high fat diet and at 10 weeks underwent ex vivo acetylcholine-mediated endothelial function assessment on isolated aortic rings.

**Results:** Vessel relaxation was significantly impaired in ApoE–/– mice versus C57BL/6. Mice supplemented with low or moderate dose nitrate showed significant improvements in vessel relaxation compared to ApoE–/– mice given the high nitrate dose or ApoE–/– mice given sodium chloride. Plasma nitrate and nitrite levels were significantly increased in all three groups fed the nitrate-supplemented water.

**Conclusion:** Low and moderate dose, but not high dose, nitrate improves vascular function in ApoE–/– mice fed a high fat diet.

**EFFECT OF RECOMBINANT PLACENTAL GROWTH FACTOR 2 ON EXPERIMENTAL PREECLAMPSIA INDUCED BY TUMOR NECROSIS FACTOR-ALPHA IN PREGNANT MICE**

Chau KK²,³, Bobek G, Lim S, Hennessy A,² Makris A²

**Aims:** To evaluate the effect of pre-emptive supplemental PLGF-2 given to mice destined for experimental preeclampsia induced by tumor necrosis factor (TNF-α) infusion.

**Methods:** C57BL/6 mice were treated with daily recombinant PLGF-2 (100 µg/kg/day, n = 9) or control (phosphate buffered saline 100 µL, n = 8) intraperitoneally from gestational day (g) 13 to 19. On g13, experimental animals received continuous infusion of TNF-α (500 ng/kg/day). Of the above animals, control (n=5) and PLGF-2 (n=6) mice had continuous blood pressure measurements by radiotelemetry via a carotid artery
transducing device inserted at least 10 days prior to timed mating. The remaining animals (control n=3) were not subject to multi-echo magnetic resonance imaging in an 11.74 Tesla spectrometer on gd 17. Animals were euthanized at gd 17 and plasma, urine and tissue were collected for analysis. Data was expressed as mean±SEM.

Results: There was no difference in blood pressure or proteomina (468±231 vs. 506±14 mg/mmol; P=0.59) between mice receiving control or PLGF-2. Serum FLI-1/PPS ratio was significantly higher in mice administered PLGF-2 (333±18.6 vs. 493±4.6; P=0.007). There was also no observed difference in T2 hysteresis/junctional zone ratio (P>0.59) in mouse placentas imaged (control, 2.20±0.11; n=18, vs. PLGF, 2.22±0.11 n=16).

Conclusions: PLGF-2 does not ameliorate features of experimental pre eclampsia induced by TNF-α alpha infusion. Contrary to expectations, serum FLI-1/PPS ratio rises with PLGF-2 treatment suggesting a potentially unfavourable effect of supra-physiological levels of PLGF prior to development of pre eclampsia.

PREDICTIVE PERFORMANCE OF ECHOCARDIOGRAPHIC PARAMETERS FOR CARDIOVASCULAR EVENTS AMONG ELDERLY TREATED HYPERTENSIVE PATIENTS

Crowdhury EK, Jennings G, Dewar E, Wing LMF, Reid CM* on behalf of the ANBP2 Management Committee

*Centre of Cardiovascular Research & Education in Therapeutics, Department of Epidemiology and Preventive Medicine, Monash University, Melbourne, Victoria, Australia; *Baker IDI Heart and Diabetes Institute, Melbourne, Victoria, Australia; *School of Medicine, Flinders University, South Australia, Australia; *School of Public Health, Curtin University, Perth, Western Australia, Australia

Background: Hypertension leads to cardiac structural and functional changes, commonly assessed by echocardiography. It is not clear which echocardiographic parameters are most predictive of future cardiovascular events among elderly treated hypertensive patients over the short or long term.

Aim: To assess the predictive performance of different echocardiographic parameters in relation to cardiovascular outcomes in elderly hypertensive patients.

Methods: Echocardiographic data from the Second Australian National Blood Pressure study were used. Participants aged ≥65 years at enrolment were followed for cardiovascular events and mortality for a median of 4.1 years (short-term) and then a further median of 6.9 years (long-term). Echocardiograms were performed at baseline to measure direct and derived parameters. Left ventricular hypertrophy (LVH) was defined using threshold values of left ventricular mass (LVM) indexed to either body surface area (BSA) or height2.7: >115/95 g/m² or >249/45 g/m² in (males and females, respectively) and ≥125 g/m² or ≥215 g/m² for (both sexes).

Results: The prevalence of LVH ranged from 33–70% among the study participants (n=679) at baseline depending on the threshold used to define LVH. Of the echocardiographic parameters, after adjusting for potential risk factors using Cox-progression proportional hazard models, only LVH defined using LVM-BSA (>115/95 g/m²) predicted cardiovascular events and mortality over the short and long-term. Participants having LVH at baseline had twice the risk (hazard ratio, 95% confidence interval) of having any first cardiovascular event over the short-term (1.96; 1.11–3.45; P=0.02) and any fatal cardiovascular events (1.96; 1.14–3.37; P=0.02) over the long-term. Among other echocardiographic parameters, LV wall thickness, LV mass, and systolic dysfunction (i.e., abnormal fractional shortening) predicted only short-term cardiovascular events.

Conclusions: In elderly treated hypertensive patients LVH identified by echocardiography based on LVM indexed to BSA (>115/95 g/m²) was a reliable predictor of future cardiovascular events and mortality.

CENTRAL-TO-BRACHIAL BLOOD PRESSURE AMPLIFICATION IN PATIENTS TYPE 2 DIABETES MELLITUS: A SYSTEMATIC REVIEW AND META-ANALYSIS OF NON-INVASIVE MEASUREMENT

Clime REP, Otahal P, Schultz MG, Fell JW, Srikanth V, Sharma JE,*

*Menzies Institute for Medical Research, University of Tasmania, Hobart, Tasmania, Australia; *School of Human Life Sciences, University of Tasmania, Launceston, Tasmania, Australia; *Stroke and Ageing Research Group, Monash Medical Centre, Department of Medicine, Southern Clinical School, Monash University, Melbourne, Victoria, Australia

Background: Brachial blood pressure (BP) may not reflect central BP due to systolic BP (SBP) amplification. Patients with type 2 diabetes mellitus (T2DM) elicit vascular irregularities that may affect SBP amplification or other central BP indices (including pulse pressure [PP], augmentation pressure [AP] and augmentation index [AIx]), but this has never been systematically assessed by comparison to individuals without T2DM.

Aim: To determine, by systematic review and meta-analysis, the magnitude and variation of central-to-brachial SBP and PP amplification, AIx and AP in patients with T2DM compared to those without.

Methods: Six online databases were searched for published studies reporting non-invasive central and brachial SBP in those with and those without T2DM. Random effects meta-analyses and meta-regression were used to analyse the studies.

Results: We identified 17 studies with a total of 2,711 patients with T2DM and 10,460 controls without T2DM. There was no significant difference in SBP amplification between groups (T2DM=10.8 mmHg, No T2DM=10.2 mmHg; pooled estimate = 0.66mmHg (95% CI −0.3 and 1.5, respectively; P=0.21), but there was a large variation in both (T2DM range = 2.0–16.5 mmHg, No T2DM range = 1.0–18.1 mmHg). In the meta-regression, duration of T2DM explained 13.6% of the variance in the pooled data (P<0.15). The difference in amplification between groups increasing by 0.3 mmHg per year of T2DM. PP amplification was not significantly different between groups (P=0.16), AP, AIx and AIx corrected for heart rate were significantly higher in T2DM (P<0.05 for all).

Conclusions: Central and brachial BP amplification was not statistically different in T2DM patients with and without T2DM, but no difference in SBP (or PP) amplification, compared to those without T2DM. However, SBP amplification is highly variable and increases with duration of T2DM, altogether confirming that central systolic loading cannot be assessed from brachial BP in patients with T2DM.

BLOOD PRESSURE RESPONSE TO RENAL DENervation IN PATIENTS WITH RESISTANT HYPERTENSION AND MULTIPLE RENAL ARTERIES

Hening DR,* Marusic P*, Walton AS*, Duval J, Head O*, Eister MD*, Schlaich MP*;

*Neurovascular Hypertension & Kidney Disease Laboratory, Baker IDI Heart & Diabetes Institute, Melbourne, Victoria, Australia; *School of Medicine and Pharmacology – Royal Perth Hospital Unit, University of Western Australia, Perth, Western Australia, Australia; *Heart Centre Alfred Hospital, Melbourne, Victoria, Australia; *Neuropomsonology Laboratory, Baker IDI Heart & Diabetes Institute, Melbourne, Victoria, Australia

Background: Renal denervation (RDN) has been demonstrated to lower blood pressure (BP) and muscle sympathetic nerve activity (MSNA) in patients with resistant hypertension (RH). Previous studies have predominantly included patients with single renal arteries bilaterally. Whether RDN is feasible, safe and effective in patients with multiple renal arteries or variable renal artery anatomy remains obscure.

Aim: To determine the efficacy of RDN in patients with RH and multiple renal arteries.

Methods: We measured 24-hour BP at baseline, 3 and 6 months after RDN in 91 patients with RH including 65 patients with single renal arteries bilaterally (Group 1), 16 patients with dual renal arteries on either one or both sides (Group 2), and 10 patients with other anatomical constellations or structural abnormalities (Group 3). MSNA was obtained in 39 out of 91 patients at baseline and follow-up.

Results: RDN significantly reduced daytime SBP in group 1 from 152±17 mmHg at baseline to 145±14 mmHg at both 3 and 6 months follow-up (P<0.001), but not in group 2: 149±12 mmHg at baseline vs. 144±16 mmHg at 3 and 6 months follow up (P=0.32); nor in group 3: 156±17 mmHg at baseline vs. 154±19 mmHg at 3 and 146±13 mmHg at 6 months follow-up (P=0.13). Resting baseline MSNA was only reduced in group 1, from 51±14 bursts/min at baseline to 45±17 bursts/min at 3 and 43±14 bursts/min at 6 months post procedure (P<0.05). There was no deterioration in kidney function in either group.

Conclusions: RDN can be performed safely in patients with RH irrespective of renal artery anatomy. The presence of single renal arteries with or without structural abnormalities is associated with a more pronounced RDN-induced reduction in BP and MSNA when compared to the presence of dual renal arteries. However, when patients with dual renal arteries underwent renal nerve ablation in all existing arteries, a greater BP reduction was observed suggesting that incomplete renal sympathetic denervation may account for differing BP responses.

SHOULD YOU LEAVE A LEGACY? POTENTIAL EFFECTS OF DELAYED BLOOD PRESSURE LOWERING PHARMACOTHERAPY IN INDIVIDUALS STRATIFIED BY ABSOLUTE CARDIOVASCULAR DISEASE RISK

Ho CLP*, Dourj J, Jackson R*, McManus RJ, Sundström J, Nelson MR*

*University of Tasmania, Hobart, Tasmania, Australia; *Bond University, Gold Coast, Queensland, Australia; *University of Auckland, Auckland, New Zealand; *Oxford University, UK; *Monash University, Clayton, Victoria, Australia; *Curtin University, Perth, Western Australia, Australia; *Uppsala University Hospital, Stockholm, Sweden

Background: Cardiovascular disease (CVD) is still the major contributor to the global burden of disease. To ensure that medication is received by those most likely to benefit from it in primary care, risk approach using CVD absolute risk (low [<10%], medium [10–15%] and high [>15%]) on all-cause and disease-specific mortality.

Aim: To investigate the effects of delayed BP lowering therapy on those with elevated BP over a spectrum of absolute risk (low [<10%], medium [10–15%] and high [>15%]) on all-cause and disease-specific mortality.

Methods: We conduct a post-hoc analysis of long-term CVD mortality and all-cause mortality in the Australian National Blood Pressure study (ANBP). The ANBP study was conducted in the 1990s on 3,427 participants aged 40–69 years who were not from the general population with mildly elevated BP and no history of CVD or diabetes. We plan to probability match all participants to the Australian Institute of Health and Welfare National Death Index,
KIDNEY TARGETED microRNA-181A MIMIC TREATMENT IN HYPERVENTILATION BPH/2J MICE

Jackson KL1, Marques FZ2, Stevenson EP3, Charach FJ4, Davem PJ4, Head GA5
1Neuropsychology Laboratory, Baker IDI Heart and Diabetes Research Institute, Melbourne, Victoria, Australia; 2School of Health Sciences, Federation University of Australia, Ballarat, Victoria, Australia

Background: BPH/2J mice are a genetic model of hypertension driven by greater activity of the sympathetic nervous system (SNS) and renin-angiotensin system (RAS). During the dark period of the 24-hour light cycle when hypertension is at its greatest, BPH/2J mice display enhanced renal renin mRNA, possibly related to lower levels of microRNA (miR-181a), which is a negative regulator of renin mRNA.

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

Methods: BPH/2J and normotensive BPN/3J control mice (n=6–10) were administered miRNA/miR-181a mimic or vehicle control (0, 1, 5, and 25 nmol/mouse) by intraperitoneal injection on 5 consecutive days, and followed daily for 24 sessions.

Results: The peak hypotensive effect of the mimic relative to vehicle treatment in BPH/2J mice was observed 12–15 h after the 5 nmol dose and kidney tissue was collected at ~50 hours for measurement of renin mRNA.

Conclusion: BPH/2J mice were randomized to an ISE or a control group. Participants underwent pre- and follow-up assessments to evaluate clinical practice by addressing clinician concerns. Such an approach has the potential to significantly reduce the number of well, symptom-free, individuals labeled as having a disease (hypertension) with attendant financial burdens (cost of drugs, monitoring and follow-up) and potential side effects.

KIDNEY TARGETED microRNA-181A MIMIC TREATMENT IN HYPERVENTILATION BPH/2J MICE

THE EFFECTS OF 8 WEEKS OF INTERVAL SPRINTING EXERCISE ON CARDIOVASCULAR FUNCTION OF OVERWEIGHT POSTMENOPAUSAL WOMEN

Liu D, Lin DP, Boucher SH, Boucher YN
School of Medical Sciences, University of New South Wales, Sydney, New South Wales, Australia

Background: The effect of interval sprinting exercise (ISE) on cardiovascular function of overweight postmenopausal women has not been determined.

Aim: To determine the effect of an 8-week ISE intervention consisting of three weekly 20-min bouts of ISE on cardiac autonomic function.

Methods: Twenty postmenopausal women (BMI 28.0±0.89 kg/m²; age 53.3±1.3 years) were randomly assigned to an ISE or a control group. Participants underwent pre- and post-training testing including an aerobic fitness test and heart rate and blood pressure variability analysis to measure autonomic influence on the heart. ISE participants undertook 24 supervised exercise sessions that involved 8 s sprints on a cycle ergometer followed by 12 s of easy pedaling, repeated for a total of 20 minutes.

Results: ISE compared to control women significantly (P<0.05) improved their aerobic fitness (2.3±0.11 vs. 1.7±0.11 L/min). Baroreceptor sensitivity of the ISE (9.3±0.68 ms/mmHg) increased significantly at post-test (P<0.05) compared to the control group (6.8±0.69 ms/mmHg).

Conclusion: Twenty minute bouts of ISE repeated over 24 sessions led to a significant improvement in aerobic fitness and a significant increase in baroreceptor sensitivity.

The Effect of Genes Involved in Monogenic Human Cardiomyopathies in a Polycystic Model of Cardiac Hypertrophy

Prestes PR1, Marques FZ2, Curl CL1, Lewandowski P1, Delbridge LMD1, Charchar FJa, Harrap SBb
1School of Applied and Biomedical Sciences, Faculty of Science and Technology, Federation University Australia, Ballarat, Victoria, Australia; 2Heart Failure Research Group, Baker IDI Heart and Diabetes Research Institute, Melbourne, Victoria, Australia; 3Department of Physiology, University of Melbourne, Melbourne, Victoria, Australia; 4School of Medicine, Deakin University, Waurn Ponds, Victoria, Australia

Background: Cardiac hypertrophy (CH) is the main risk factor for heart disease after a certain age. We wondered whether genes implicated in monogenic forms of human CH might also be involved in the more common polycystic forms of the disease.

Aim: To use the hypertrophic heart rat (HHR), a unique normotensive polycystic model of CH, to identify mRNA expression of genes associated with monogenic forms of dilated and hypertrophic cardiomyopathy in humans.

Methods: We measured the expression of 37 transcripts with the TruSeq Targeted RNA expression kit using the MiSeq Desktop sequencer (Illumina) in left ventricles of HHR and its matched control strain, the normal heart rat (NHR), at five ages (2 days old, 4, 12, 33, and 50 weeks old).

Results: We found only one gene (Th) that was differentially expressed in all age groups (FDR<0.1; P≤0.05). Th is involved in cardiac amyloidosis, infiltrating cardiovascular structures, leading to hypertrophy. In rats older than 13 weeks old, we found expression of 4 genes (Acb1, Ankrd1, Cav1 and Fh2) was upregulated in the HHR. The proteins encoded by these genes are involved in a variety of muscle development pathways, growth and contractility. Interestingly, Ankrd1 (fold change 1.3–2.5) has been found to be upregulated in the failing myocardium of dogs and in the left ventricles of patients with CH. Fh2 is associated with cardiomyopathy in rats, but seems to not be essential for cardiac development in mice.

Conclusion: Our results show that genes involved in monogenic forms of human CH may also influence polycystic forms of the disease and thus merit further investigation.

BARORECEPTOR SENSITIVITY IN DIABETIC RATS WITH TREATED AND UNTREATED HYPERTENSION

Ramachandran H, Salum E, Kampus P, Kals J, Town G, Avolio AP, Butlin M
1Faculty of Engineering, Macquarie University, Sydney, New South Wales, Australia; 2Department of Cardiology, University of Tartu, Tartu, Estonia; 3Faculty of Medicine and Health Sciences, Macquarie University, Sydney, New South Wales, Australia

Background: Diabetes is associated with raised blood pressure (BP) and cardiovascular risk. Baroreceptor sensitivity (BRS) is a feedback mechanism controlling spontaneous changes in BP and decreases with age and in different diseases.

Aim: To quantify BRS in spontaneous changes in BP in diabetic rats and rats treated with antihypertensive therapy.

Methods: Male Wistar rats (aged 6 weeks) were divided into control (n=8), control with antihypertensive treatment (control+Tx, telmisartan, 10 mg/kg/day; n=5), induced diabetic rats (diabetes, 1.25±0.29 mmHg; P=0.95) or diabetes with antihypertensive treatment (diabetes+Tx; n=8). At 18 weeks, rats were anesthetized (urethane, 1.3 g/kg) and an electrocardiogram performed and aortic BP was measured (1.2 F solid-state pressure tipped catheter, introduced via the femoral artery). BRS was quantified using custom-written scripts to detect sequences of at least 3 pulses with a minimal systolic BP change of 1 mmHg and minimum R-R change of 1 ms.

Results: Both control (142±16 mmHg) and diabetic (132±22 mmHg) rats were hypertensive. Anti-hypertensive treatment successfully lowered systolic BP (control+Tx 105±11 mmHg; diabetes+Tx 119±14 mmHg). Antihypertensive treatment did not alter BRS for either controls (0.87±0.45 ms/mmHg vs. control+Tx 0.88±0.33 ms/mmHg; P=0.99) or diabetic rats (diabetes 1.25±0.29 mmHg vs. diabetes+Tx 1.46±1.04 mmHg; P=0.56). There was also no difference between diabetic rats and controls (P=0.08) or those with antihypertensive treatment (P=0.25).

Conclusions: Despite altering BP through antihypertensive treatment, BRS measured through spontaneous changes in BP, was unchanged for both control and diabetic animals.

INHIBITING MITOCHONDRIAL FUSION WITH MVDI-1 IMPROVES SURVIVAL OF HUMAN CARDIAC RESIDENT STEM CELLS

Rosdiah MA1, Sivakumaran P, Delbridge LMD, Lim SY2,3
1O’Brien Institute Department, St. Vincent’s Institute, Melbourne, Victoria, Australia; 2Department of Physiology, University of Melbourne, Melbourne, Victoria, Australia; 3Department of Surgery, University of Melbourne, Melbourne, Victoria, Australia

Background: Stem cell therapy is a promising approach to treat myocardial infarction. However, survival of transplanted cells is poor due to the hostile environment of the infarcted heart. Therefore, novel strategies are needed to improve the survival of stem cells post-transplantation. Mitochondria are morphologically dynamic organelles constantly undergoing...
fission and fusion, processes essential to maintain organelle function and cell viability. Inhibiting mitochondrial fission has been shown to promote survival of several cell types. However, its role in survival of human cardiac resident stem cells (CRSCs) remains unknown. Aims: To determine whether Mdivi-1, an inhibitor of mitochondrial fission protein DRP1, can improve survival of a novel population of human CRSCs.

Methods: WB2B+ CRSCs were isolated from human atrial appendages. In an oxidative stress injury model, cells were treated with 3 μM of H2O2 for 3 hours. Induction of cell death was measured using an amidolytic assay.

Results: Mdivi-1 significantly reduced H2O2-induced cell death at 50 μM and 100 μM (P<0.05 vs. vehicle; n=6). This cytoprotective effect was accompanied by an increased proportion of cells with tubular mitochondria (P<0.0001 vs. vehicle; n=3–5), but independent of mitochondrial membrane potential reduction and reduction of ROS production. In the SIRI model, pre-treatment with 5 μM Mdivi-1 for 2 hours and co-treatment with 10 μM Mdivi-1 significantly reduced cell death (P<0.05 vs. vehicle; n=8). However, post-treatment with Mdivi-1 during reperfusion did not significantly affect cell survival.

Conclusion: Inhibition of mitochondrial fission with Mdivi-1 can promote survival of human WB2B+ CRSCs, and may therefore be employed to enhance the therapeutic efficacy of post-transplanted CRSCs in the infarcted myocardium. The lack of mitochondrial membrane potential recovery and ROS reduction might suggest a novel mechanism of protection by Mdivi-1, although requires further investigation.

PREVENTORS OF MORTALITY IN NEWLY DIAGNOSED HEART FAILURE PATIENTS: A MATCHED NESTED CASE-CONTROL STUDY

Sahie B1, Owen AJ2, Reid CM1,2

1Department of Epidemiology and Preventive Medicine, Monash University, Melbourne, Victoria, Australia; 2School of Public Health, Curtin University, Perth, Western Australia, Australia

Background: Despite advances in therapeutics, the prognosis of patients with heart failure (HF) remains poor. The etiology and clinical course of HF varies significantly, and, importantly, the effect of risk factors on prognosis also varies according to the spectrum of the syndrome and patient characteristics.

Aims: To identify the long-term predictors of mortality in hypertensive patients with newly diagnosed HF.

Methods: A matched case-control study, nested within the Second Australian National Blood Pressure Study (ANBP2) and ANBP2 post-final follow-up was undertaken. Case subjects were HF patients diagnosed after enrolment in the study and who had died during the follow-up. Controls subjects were 1:1 matched to cases based on age (5 year range), sex and calendar year. A total of 147 cases and their 147 randomly matched controls were included in the analysis. Adjusted odds ratio (AOR) and 95% confidence interval (CI) was estimated to identify predictors of mortality using multiple conditional logistic regressions.

Results: Mortality was associated with pre-existing diabetes (AOR=2.17, 95% CI 1.31–7.87; P<0.01), impaired renal function (AOR=2.03, 95% CI 1.07–3.96; P<0.03) higher systolic BP (AOR=1.03, 95% CI 1.01–1.05; P=0.01) and current smoking (AOR=3.62, 95% CI 1.11–11.8; P=0.03). However, neither diastolic BP (AOR=0.99; 95% CI 0.96–1.02; P=0.65), overweight (AOR=0.85; 95% CI 0.45–1.58; P=0.60) nor obesity (AOR=1.13, 95% CI 0.55–2.35; P=0.74) were significantly associated with mortality.

Conclusions: In newly diagnosed HF patients, comorbidities, elevated systolic BP and current smoking were associated with mortality, while diastolic BP overweight and obesity were not.

THE ROLE OF TISSUE PLASMINOGEN ACTIVATOR IN BLOOD PRESSURE REGULATION FOLLOWING STRESS

Trang EP1, Jackson KL2, Sashindranath M2, Stevenson ER3, Johns EMC2, Davern PJ2, Mdivi-1 significantly reduced H2O2-induced cell death at 50 μM and 100 μM (P<0.05 vs. vehicle; n=6). This cytoprotective effect was accompanied by an increased proportion of cells with tubular mitochondria (P<0.0001 vs. vehicle; n=3–5), but independent of mitochondrial membrane potential reduction and reduction of ROS production. In the SIRI model, pre-treatment with 5 μM Mdivi-1 for 2 hours and co-treatment with 10 μM Mdivi-1 significantly reduced cell death (P<0.05 vs. vehicle; n=8). However, post-treatment with Mdivi-1 during reperfusion did not significantly affect cell survival.

Conclusion: Inhibition of mitochondrial fission with Mdivi-1 can promote survival of human WB2B+ CRSCs, and may therefore be employed to enhance the therapeutic efficacy of post-transplanted CRSCs in the infarcted myocardium. The lack of mitochondrial membrane potential recovery and ROS reduction might suggest a novel mechanism of protection by Mdivi-1, although requires further investigation.

HYPERTENSION PREVALENCE AND BLOOD PRESSURE VARIABILITY AMONG CHILDREN AGED 5 TO 17 YEARS OLD: RESULTS FROM THE 2011–2013 AUSTRALIAN HEALTH SURVEY

Veloudi P1, Blizzard L2, Velandaa K3, Schutzm GD, Sharmam J4

1Monash University, Melbourne, Victoria, Australia; 2Department of Epidemiology and Preventive Medicine, Monash University, Melbourne, Victoria, Australia; 3School of Public Health, Curtin University, Perth, Western Australia, Australia

Background: Blood pressure (BP) screening in children is not a routine clinical assessment, partly due to concerns about diagnostic accuracy and controversy over normative reference BP values. Clinicians may be reluctant to interpret abnormal BP readings if they do not know the child’s long-term BP variability. This is particularly important in the young, as BP values. Clinic BP accuracy in children is thought to be compromised by increased BP variability (BPV) and falsely elevated readings which normalize over repeated measurements. However, these issues have not been investigated.

Aims: To determine the prevalence of hypertension and the role of BP among the largest population study of children in Australia to date.

Methods: Two consecutive BP measurements were recorded by oscillometry in 3,047 children (aged 12 years; 95% CI: 12.13, males 52%) from the 2011–2013 Australian Health Survey. A 3rd BP reading was taken if the difference between the first and second SBP readings (ΔSBP) was ±10 mmHg. ΔSBP was calculated as the coefficient of variation (CV, [SD/mean SBP] x 100) for children with three SBP readings.

Results: The prevalence of hypertension, as defined according to age, sex and height referenced BP values, was 4.0% and this was significantly greater in overweight compared with non-overweight children (7.3% vs. 3.6%). From the first to second measurements, SBP decreased in 58%, did not change in 10%, and increased in 32% of the population. The strongest independent correlates of SBP were sex (β±0.01; P=0.03), height (β±0.19; P=0.001), mass body index (BMI; β±0.61; P<0.001), serum vitamin B12 (β±0.007; P=0.005), serum ferritin (β±0.018; P<0.01) and urine sodium concentration (β±0.03; P=0.023). BPV and ΔSBP were significantly higher among children with hypertension compared to children with normal SBP (13.1 mmHg [95% CI: 9.7, 16.5] vs. 7.8 mmHg [95% CI: 7.5, 8.3]; P=0.004 and ΔSBP: 13.8 mmHg [95% CI: 9.5, 18.4] vs. 6.4 mmHg [95% CI: 6.0, 6.7]; P<0.001). Minimum ΔSBP corresponded to normal SBP ranges irrespective of age.

Conclusions: Hypertension prevalence is 4% among Australian children, and is associated with increased BP, increased BMI, and nutrient biomarkers. Importantly, BPV does not necessarily normalize over repeated measurements and the magnitude of BPV has clinical relevance with respect to hypertension diagnosis.

CHANGES IN INTRARENAL CATECHOLAMINES IN DIABETES AND HYPERTENSION

Watson AMD5, Gould EAM6, Jackson KL6, Moretti J6, Lambert GW6, Head GA6, Jordeleit-Dahn KM6

1Baker IDI Heart and Diabetes Institute, Melbourne, Victoria, Australia; 2Central Clinical School, Department of Medicine, Monash University, Melbourne, Victoria, Australia

Background: We and others have found greater levels of in renal noradrenaline (NA) content in hypertensive rodents as compared to normotensive controls. Using the hypertensive SHR mouse model, we have previously found greater cortical tubular staining for the neural marker tyrosine hydroxylase (TH) in kidneys of hypertensive BPH/2J Schaefer mice. Changes in intrarenal nerves in diabetes have not, however, been investigated previously.

Aims: To investigate the effect of diabetes on neural staining and catecholamine content in kidneys of Schaefer mice with BPH/2J and without BPH/2J concomitant hypertension.

Methods: After 10 weeks of study, hypertensive BPH/2J and normotensive BPH/2J Schaefer mice with and without concomitant streptozocin-induced diabetes (55 mg/kg, i.p.) were placed in metabolic cages for 24 h, after which their kidneys were collected for analysis. In a separate group of mice BP telemetry probes were implanted.

Results: Induction of diabetes did not change the hypertensive status of BPH/2J mice (mean arterial pressure: 135±1 vs. 131±3 mmHg for non-diabetic vs. diabetic BPH/2J mice, respectively, n=3 per group). Diabetic mice showed significantly greater albuminuria, with diabetic hypertensive animals showing significantly greater albuminuria than normotensive diabetic animals. Glomerular mesangial expansion was significantly greater in diabetic mice compared to respective controls, with no difference between hypertensive and normotensive diabetic mice. Similarly, plasma cystatin C was significantly lower in diabetic mice, with no difference between hypertensive and normotensive diabetic mice. NA and dopamine levels were significantly greater in hypertensive mice, but interestingly normotensive and hypertensive diabetic
mice had significantly less NA and dopamine levels compared to mice with hypertension alone. Hypertensive mice had significantly more cortical tubular TH staining than normotensive mice. This was not, however, seen in diabetic hypertensive mice.

**Conclusion:** The present data indicate that diabetes alters renal nerve density and distribution in a manner which is independent of hypertensive status. The findings suggest that diabetes alters neural function in the kidney.

**ADRENOCORTICOTROPIC HORMONE ADMINISTRATION IMPROVES THE DIAGNOSTIC PERFORMANCE OF ADRENAL VEIN SAMPLING**

Wolley MJ, Ahmed AH, Gordon RD, Stowasser M

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

**Background:** Adrenal vein sampling (AVS) is vital for determining treatment options for primary aldosteronism (PA), but is a difficult procedure. Successful cannulation depends on correct catheter placement, judged by adrenal to peripheral vein cortisol gradients of ≥3. Aldosterone/cortisol (A/F) ratios are then calculated to correct aldosterone concentration for dilution from non-adrenal blood, and comparisons are made between left, right and peripheral A/F ratios to determine if the disease is unilateral. Adrenocorticotropic hormone (ACTH) infusion or bolus has been reported to improve AVS success rates by increasing cortisol secretion, but effects on aldosterone and thus laterisation are controversial.

**Aim:** To determine the effects of ACTH administration on AVS in regard to success rates and lateralization of PA.

**Methods:** AVS was performed in the morning after overnight recumbency in patients with PA confirmed by fludrocortisone suppression test. After bilateral sequential sampling, the catheters were withdrawn and a bolus of 250 µg of ACTH was given. After 15 minutes bilateral sampling was repeated.

**Results:** From 45 AVS procedures 413 samples were obtained; 214 pre-ACTH and 199 post-ACTH. The mean peripheral cortisol increased from 14.9 to 26 (P=0.001) with ACTH. Pre-ACTH, 76/91 (83.5%) left samples were adequate (cortisol gradient ≥3), improving to 86/90 (95.6%) post-ACTH (cortisol gradient ≥5) (P=0.014). Pre-ACTH 83/98 (84.7%) right samples and post-ACTH 85/92 (92.4%) were adequate (cortisol gradient ≥3). Of these 33, 21 were bilateral and 9 unilateral both before and after ACTH. Two further cases that appeared bilateral before ACTH were unilateral after ACTH (one surgically operated and cured and one treated medically), and one was unilateral before ACTH and bilateral afterwards (treated medically with good result).

Nine cases had a non-diagnostic study pre-ACTH but a diagnostic study post-ACTH, of which 3 were unilateral and 6 were bilateral (33/45 diagnostic studies pre-ACTH vs. 42/45 post-ACTH; P=0.02).

**Conclusions:** ACTH improved cortisol gradients and AVS success, resulting in an improved proportion of diagnostic studies. There was a low proportion of discordance between pre- and post-ACTH diagnoses, suggesting that ACTH is unlikely to confound lateralization.

**OBSTRUCTIVE SLEEP APNOEA IS COMMON IN PATIENTS WITH PRIMARY ALDOSTERONISM AND IMPROVES WITH ADRENALECTOMY OR MINERALOCORTICOID RECEPTOR ANTAGONISTS**

Wolley MJ, Cowley Da, Ahmed A, Gordon RD, Stowasser M

*Endocrine Hypertension Research Centre, University of Queensland School of Medicine, Greenslopes and Princess Alexandra Hospitals, Brisbane, Queensland, Australia; bDepartment of Nephrology, Royal Brisbane and Women’s Hospital, Brisbane, Queensland, Australia*

**Background:** Obstructive sleep apnoea (OSA) commonly co-exists with primary aldosteronism, particularly in the setting of resistant hypertension. The exact nature of the relationship is unclear, but evidence from other patient groups suggests that states of fluid and sodium retention can increase upper airway resistance and contribute to sleep apnoea. It is unclear, however, if treatment via mineralocorticoid receptor blockade, or adrenalectomy (for aldosterone producing adenoma, APA), improves OSA parameters in these patients.

**Aim:** To determine if specific medical or surgical treatment of primary aldosteronism improves OSA, as measured by the apnoea hypopnea index (AHI).

**Methods:** Patients undergoing diagnostic workup for primary aldosteronism were recruited if they had any symptoms suggestive of OSA (significant snoring, witnessed apnoeas, morning fatigue/ headaches, daytime somnolence). Patients with confirmed primary aldosteronism underwent polysomnography (PSG) at baseline and again at least 3 months after specific treatment for primary aldosteronism. Patients with severe OSA were referred for continuous positive airway pressure (CPAP) and only restudied with PSG if this had not yet commenced at the planned time of restudy.

**Results:** Of 34 patients with primary aldosteronism who were screened, 7 (21%) had no evidence of OSA (AHI <3), and 9 (26%) had mild (AHI 3–15), 8 (24%) moderate (AHI 15–30) and 10 (29%) severe OSA (AHI >30). 20 patients had repeat PSG performed ≥3 months after treatment for primary aldosteronism (mineralocorticoid receptor antagonists in 13 with bilateral PA and adrenalectomy in 7 with unilateral PA). In this group the median AHI reduced from 22.5±14.7SD to 12.3±12.1SD (P=0.018). The AHI fell in 15 patients (10 bilateral and 5 unilateral) and remained the same or increased in 5 (3 bilateral and 2 unilateral). There was no significant change in median patient weight (95.9 kg vs. 98.5 kg; P=0.034; mean change +0.66 kg). A small but significant reduction in neck circumference occurred, however (41.6 cm vs. 41.2 cm; P=0.012; mean change –0.56cm).

**Conclusions:** Obstructive sleep apnoea is a common finding in patients with primary aldosteronism, and improves with specific therapy for this condition. Aldosterone and sodium-mediated fluid retention in the upper airways and neck region may be a potential mechanism for this relationship.
Abstracts From the 37th Annual Scientific Meeting of the HBPRCA

Hypertension. 2016;67:e7-e21
doi: 10.1161/HYP.0000000000000042

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2016 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/67/5/e7

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/