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HBPRCA Oral Presentations

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


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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 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 when:

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 levels of bilaterally activated microglia 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 STREPTOZOTOCIN-TREATED RATS VIA ACTIVATION OF MICROGLIA IN CENTRAL CARDIOVASCULAR CONTROL CENTRES

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Background: We have previously reported that streptozotocin (STZ)-treated hyperglycemic rats display activation of microglia, the brain’s local inflammatory cells, within the paraventricular nucleus (PVN) and other cardiovascular centres 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 reports suggest that drinking 1% saline, which might ameliorate dehydration, could cause hypertension by 2 weeks after STZ treatment.

Methods: The aim of this study was to determine whether or not microglial activation contributes to saline-induced hypertension. STZ-treated rats were subjected to microglial activation on the PVN of STZ diabetic rats and to determine whether or not microglial activation contributes to saline-induced hypertension.

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

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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 were not (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 90 mL/min). Twenty four percent of patients had eGFR ≤ 60 mL/min; 48% had eGFR = 30–59 mL/min and 28% had eGFR > 90 mL/min. At calibration in the clinic, there was no significant difference in systolic readings obtained by clinic or home monitor, the mean difference in systolic blood pressure being 3.5 mmHg lower (95% CI −8.0 to 1.9; P = 0.04; 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 (46%); extra readings (41%); and non-verifiable BP readings (11%). Clinic measurements suggested that 47% of the patients who had controlled BP (systolic <140 mmHg), whereas HBPM suggested that 37% of the patients had controlled BP (systolic ≤135 mmHg; χ² = 4.0, P = 0.05 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

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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, kidney function, and other key variables were collected.

Results: A representative sample of 343 rural households (87% response rate) yielded 194 hypertensive individuals (hospital based and self-reported). Among those identified hypertensive, 83% were aware of their condition, 45% on treatment, and 37% controlled. Factors significantly associated with awareness, treatment, and control were age (P < 0.05), education (P < 0.05), and income (P < 0.05). In a multivariate analysis, the strongest predictor of awareness and treatment was education (OR: 1.9, 95% CI: 1.1-3.1 and OR: 2.1, 95% CI: 1.1-3.9, respectively).

Conclusions: Education is a strong predictor of awareness and treatment of hypertension in rural Indian populations. Efforts should be directed towards increasing education of the population with regard to hypertension, especially in the primary health care setting.
BLOOD PRESSURE MANAGEMENT – ISOMETRIC HANDGRIP EXERCISE REDUCES HYPERTENSION

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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 may be potentially 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 group effect 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 diet or 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 anti-hypertensive medication (diabetes). 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 to 123.1±2.3 mmHg (P=0.007), was seen, while in the 5% MVC group a reduction of -5 mmHg, from 125±11.7 to 120±15.1 mmHg (P=0.033), 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 74±6.6 to 68±9.1 (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.

MACROPHAGE-DERIVED INSULIN-LIKE GROWTH FACTOR 1 CONTRIBUTES TO VASCULAR FIBROSIS, AORTIC STIFFENING AND ELEVATED BLOOD PRESSURE IN HYPERTENSIVE MICE


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Background: M2 macrophages accumulate in the vessel wall during hypertension and are important mediators of vascular remodelling, fibrosis and stiffening. However, the mechanisms involved are yet to be defined. M2 macrophages are an important source of insulin-like growth factor 1 (IGF-1). In other disease settings, IGF-1 is known to contribute to fibrosis and tissue growth but its role in vascular remodelling during hypertension remains unknown.

Aims: To examine whether macrophage-derived IGF-1 contributes to vascular fibrosis, aortic stiffening and elevated blood pressure (BP) in hypertensive mice.

Methods and Results: In 10–12 week old male C57BL/6J mice, chronic infusion of angiotensin (Ang) II (0.7 mg/kg/d for 14 days, s.c.) elevated systolic BP (measured by tail-cuff) by >50 mmHg (P<0.05) and increased collagen content (Masson’s trichrome) and stiffness (ultrasound measurement of pulse wave velocity) of the aorta, each by ~2-fold (P<0.05). These changes were accompanied by a 6.6-fold increase in numbers of M2 macrophages (CD45+CD11b+Ly6g4/60/2020+ cells) in the aortic wall (P=0.005), and, importantly, expression of the M2 macrophage marker, CD206, correlated strongly and positively with that of IGF-1 (r²=0.57, P<0.001). Depletion of circulating monocytes (precursors of tissue macrophages) by treatment of mice with clodronate-containing liposomes (50 mg/kg, every 3 days, i.v.) reduced the Ang II-dependent influx of M2 macrophages into the aorta by 75% (P<0.05) and simultaneously inhibited angiogenic IGF-1 expression by 35%, collagen deposition by 34%, aortic stiffening by 43% (P<0.05), and systolic BP by 25 mmHg (P<0.05). Finally, macrophage-specific IGF1-deficient mice (LysMCre/+ x IGF-1fl/fl) that were treated with Ang II displayed reduced IGF-1 expression in the vessel wall (by ~33%) compared to similarly treated control mice (LysMCre/+ x IGF-1+/+) (P<0.05). Moreover, the IGF-1 deficient animals were protected from Ang II-induced increases in aortic collagen deposition, aortic stiffening and systolic BP (all P<0.05).

Conclusions: These results demonstrate that M2 macrophage-derived IGF-1 plays a crucial role in the aortic fibrosis that contributes to vascular stiffening and elevated systolic BP during Ang II-induced hypertension in mice. Thus, future studies aimed at unraveling the cellular targets and second messenger pathways activated by IGF-1 in the aortic wall have the potential to reveal new targets for novel antihypertensive therapies.

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

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Background: The prevalence of hypertension increases with age. Chronic low-grade inflammation commonly occurs with aging, and inflammasomes are important initiators of inflammatory responses. We tested whether aged mice exhibit an enhanced pressor response to angiotensin II (Ang II) and whether this is associated with inflammation, enhanced vasoconstriction 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 + MCC950 (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-33, CC2R, COL7 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 Nox2-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: 139±7 vs. 145±10 mmHg; n=6–7; P>0.05).

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

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

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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 leucocytes. Stem cells offer great therapeutic potential for stroke patients and may improve stroke outcome via multiple mechanisms. Human amnion
**Aim:** To assess, in healthy young adults, systolic blood pressure (SBP) responses to standing before and after GTN and their 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 6G 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, falling to 73±20.1 mmHg on initial standing and stabilizing at 109±16.9 mmHg after 2 minutes 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 resting 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.

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**CARDIAC REPAIR BY DIFFERENTIATION AND MATURATION OF CARDIOMYOCYTES FROM HUMAN INDUCED PLURIPOTENT STEM CELLS:**

**The Benefits of Short- and Long-Term Electrical Stimulation.**

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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 (EST) might affect cardiogenesis of human iPSCs.

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

**Methods and Results:** Acute EST (alternating current, charge-balanced biphasic pulse, 1 ms pulse width, 1 Hz frequency) at 200 V/mm for 5 min increased the percentage of beating embryoid bodies (EBs, 11.2±2.5% vs. 5.2±1.5% in control, non-stimulated; P<0.03; n=11–15) and gene expression of cardiac-specific contractile muscle markers ACTC1, TNNT2, MYH7 and MYL7 (P<0.05). Beating EBs displayed cyclic changes in intracellular calcium ion and chromatophoric responsiveness to isoproterenol and carbamylcholine. Chronic EST at 200 V/mm for 7 days significantly increased the percentage of cardiomyocytes with organized sarcomeres (39.8±8% vs. 23.1±11%; P<0.05; n=3), aligned in parallel with the electric field (10.1±1% vs. 6.2±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 stimulation periods are currently being evaluated. In addition, using a bioporic approach, EST is now being applied locally to cardiomyocytes derived from iPSCs in an in vivo system in rat tissue engineering chambers.

**Conclusion:** Brief EST modestly enhanced cardiac differentiation of human iPSCs. Chronic EST 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?**

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**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 presser response to stress. Allopregnanolone (alloP) is a positive allosteric modulator of the GABA receptor. We have shown that this can reduce blood pressure (BP) in adult genetically hypertensive (BPH/2J) mice.

**Aim:** To determine whether treatment of young BPH/2J mice with alloP can suppress the development of hypertension.

**Methods:** Six-week-old BPH/2J (n=19) and normotensive BPN/3J (n=15) mice were treated with a subcutaneously implanted pellet that continuously infused alloP (5-7mg/kg/day) or vehicle for some time (up to 10 weeks). Treatment with alloP was then either continued or ceased for two further weeks. After both eight and ten weeks of treatment, BP over 24 hours and cardiovascular responses to stress were measured via radio-telemetry. Levels of neuronal activity were examined using Fos immunohistochemistry.

**Results:** BP/H2J mice treated with alloP had lower BP than vehicle treated mice (–8.8±0.3 mmHg, P<0.05; n=11–15) which ameliorated 36% of their hypertension (P<0.05; n=3), aligned in parallel with the electric field (10.1±1% vs. 6.2±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 stimulation periods are currently being evaluated. In addition, using a bioporic approach, EST is now being applied locally to cardiomyocytes derived from iPSCs in an in vivo system in rat tissue engineering chambers.

**Conclusion:** Brief EST modestly enhanced cardiac differentiation of human iPSCs. Chronic EST 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.

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

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**Background:** Insulin-responsive aminophidase (IRA) is present in high concentrations in the kidney, particularly principle cells of the collecting duct. Vasoressin, a substrate of IRA, induces the translocation of AQP-2 to the apical membrane of principal cells of collecting ducts to increase water reabsorption. Previous studies found that IRA–/– mice have double the plasma vasopressin (consistent with reduced breakdown) and double the renal AQP-2 protein levels compared to WT mice.

**Aim:** To determine the functional significance of IRA on sodium and water homeostasis.

**Methods:** 8-month-old male IRA–/– and IRA wild-type (WT) mice (n=12 per group) were placed in metabolic cages to collect 24 hour urine samples. Urinary excretry profile was examined at baseline and during 24 h of water deprivation. At 17 months GFR was measured (FITC-sinistrin clearance) and a subset of mice (n=5 per genotype) were implanted with radiotelemetry devices to measure basal arterial pressure. Mice were then placed on a high salt (5% NaCl) diet and arterial pressure, GFR and 24 h urine excretion measurements were repeated.

**Results:** 24 hour urine excretion and water intake of IRA–/– mice were ~25% less (P<0.01), and urinary osmolality was greater, than WT mice. Water deprivation led to a ~50% reduction in urine excretion, and increased urinary osmolality, a response that was not different between genotypes. Chronic high salt diet increased sodium excretion 11-fold in both groups. Water intake and urine excretion were markedly increased on high salt (P<0.001). However, the increase in both was less in IRA–/– mice (P<0.05). MAP was not different between genotypes, but FITC-sinistrin t1/2 was significantly lower and calculated GFR was significantly greater in IRA–/– compared to WT mice (17±3±2 µL/min/100 g body weight). High salt increased MAP by ~12 mmHg and reduced GFR in aged mice, but these changes were similar for each genotype.

**Conclusions:** The absence of IRA did not impact the ability of mice to concentrate urine in response to water deprivation. Nor did the absence of IRA impede the ability of IRA–/– mice to excrete a sodium load in response to a high salt diet. In conclusion, IRA–/– mice maintain sodium and water homeostasis at lower levels of water intake and excretion. Further IRA–/– may be protected from age-associated decline in GFR.

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

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**equal contribution**

**Background:** The hereditary lineage of the Y chromosome is an integral determinant of cardiovascular disease risk in males. Y chromosome lineage accounts for a 15–20 mmHg difference in arterial pressure as where the Y chromosome is deleted in male mice (Shi et al, 2015). The genotype of mouse is a major factor affecting the stroke-prone spontaneously hypertensive rat (SHRSP) is replaced with the normotensive Wistar Kyoto (WKY) Y chromosome (SP/ WKY) and vice versa (WKY SP/y). 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 for acetylcholine: SHRSP: 7.3±0.1 vs. WKY: 7.9±0.1; P<0.01). Replacing the SHRSP Y chromosome with the normotensive WKY Y chromosome (EC50: SP/WKY: 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 x104 cells; P<0.05), and introgression of the alternate Y chromosome reduced infiltration (SP/WKY: Y 8.3±0.7 x104 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 in aortas from all four strains with anti-CD3 and anti-CD28 antibodies worsened endothelial function only in SHRSP aortas (Rmax untreated: 72±9% vs. T cell stimulated: 39±5%; P<0.05). Importantly, inhibition of constrictor prostanoid production reversed T cell-stimulated vascular dysfunction in the SHRSP. Scavenging of reactive oxygen species (ROS) also abrogated vascular dysfunction and prevented elevated constrictor prostanoic production in T cell-stimulated SHRSP aortas, highlighting a close interaction between ROS and cyclo-oxidase activity.

**Conclusion:** Y chromosome lineage influences immune-mediated vascular dysfunction via a prostanoic-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**

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**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) 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.

(ii) 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 VENTROMEDIAL HYPOTHALAMUS

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Background: Obesity during pregnancy is associated with a greater risk of developing hypertension in the offspring. Plasma leptin levels correlate strongly with blood pressure hypertension in the offspring. 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) signaling 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 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 l)

Results: mHFD rabbits exhibited higher MAP and RSNA than mCD rabbits (P<0.05). α-MSH injection increased MAP (10%), 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 l)

Conclusion: Exposure to over-nutrition during development alters the leptin and MC signaling pathway in the VMH of offspring that were born from obese mothers.

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

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Background: Hypertension is a chronic inflammatory disease, with the kidneys being a major site of inflammation. We have shown that inflammation and interleukin-1, beta (IL-1β) production is 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 in mice with established one-kidney (1K)/DOCA/salt-induced hypertension.

Methods: Hypertension was induced in male C57BL/6J mice by uninephrectomy, treatment with deoxycorticosterone (DOCA; 2.4 mg/d, s.c.) and replacement of drinking water with saline (1/10 DOCA/salt). Control mice were uninephrectomized and received a placebo pellet. Ten days post-surgery, mice were randomized into a vehicle (0.9% saline, i.p.) for 12 days prior to being killed. Systolic BP was measured by tail cuff and kidneys were harvested for real-time semi-quantitative PCR and flow cytometric analysis to measure expression of inflammatory markers and collagen, as well as immune cell infiltration. Renal collagen content/fibrosis was also assessed by measuring hydroxyproline content. Renal damage was assessed by calculating kidney/body weight ratio 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), yet surprisingly this was not accompanied by a decrease in immune cell infiltration (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<0.01) 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 effects of anakinra are regulated by protective actions in other BP regulating organs such as the arteries and brain.

TRANSCARDIAC GRADIENT OF CARDIO-microRNAs IN THE FAILING HEART

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Background: Circulating cardio-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 left ventricular 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-16-5p, 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-140, miR-393b-3p, miR-23c-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-16-5p, miR-17-5p, miR-27a-3p, miR-30a-5p, miR-30d-5p, miR-30e-5p, miR-130a-5p, miR-140-5p, miR-199a-5p and miR-451a. The transcardiac gradient of microRNAs in heart failure targeted pathways related to heart disease, including extracellular-matrix receptor interaction, transforming growth factor beta signaling pathway, and cytokine-cytokine receptor interaction.

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 pathophysiology of heart failure, since they are involved in pathways related to disease progression, including fibrosis.

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

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Background: Noradrenaline 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 (T) in the 3’ untranslated region (UTR) of the mRNA of the NET is associated with diseases associated with NET dysfunction, and to elucidate the mechanism involved.
miR-19a-3p in T tension. This might be explained by the presence of a binding site for the microRNA Ishizaki T, Gondo Y, Rakugi H, Willcox B, Newton-Cheh C, Seto T, Willcox DC, Masaki K, Kamide K, Ryuno H, Oguro R, Burns School of Medicine, University of Hawaii, Honolulu, Hawaii, USA; cBasic & Clinical Medical Center, Honolulu, Hawaii, USA; bDepartment of Geriatric Medicine, John A. Sydney, Sydney, New South Wales, Australia; dCalifornia Pacific Medical Center Research 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 ischemia. Evidence that inhibition of ASIC1a might be neuroprotective following stroke was previously obtained using psalmotoxin (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 focused recruitment 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 mm³ in vehicle-treated SHR to 32±10 mm³ in PcTx1-treated SHR (P<0.05) and reduced striatal infarct volume from 39±6±5 mm³ (vehicle) to 24±2±8 mm³ (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.
Aim: To determine the BP effect of commonly used antihypertensives during anesthesia in an animal model of chronic kidney disease.

Methods: Blood pressure was measured during anesthesia in 6 pregnant baboons (Papio hamadryas) with experimental preeclampsia (PE). BP was measured before and after renal denervation (RDN) in the left renal artery during anesthesia using a MTT assay at 24, 48 and 72 h post-treatment.

Results: A marked increase in GluGlu-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 potential 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

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Background: Studies show that autonomic dysfunction relates to poor prognosis and rate of progression of 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 vasoconstriction, 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 group median value of 9.98) or high BRS (those above the median). Electrocardiograms, time-based HRV and power spectral 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±12 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±1 in NET; P<0.01). The mean BRS (mmHg/s) were 13.8±6.6 in ET vs. 10.3±5.2 in NET (P<0.007; 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 650±73 in ET vs. 590±1361 in ET (P=0.01). Mean multiple sclerosis severity score (MSSS) values were 2.3±1.5 vs. 2.5±1.9 (P=not significant) in ET vs. NET, respectively.

Conclusions: Intrinsic BRS measures were studied by ET vs. NET subjects were measured earlier 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 HYPERTENSION SHEEP WITH CHRONIC KIDNEY DISEASE IMPAIRS RESPONSES TO HEMORRHAGE

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

Aims: To examine the consequences of cCNDN (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 (CND).

Methods: Sheep with established hypertension and renal dysfunction (CND group) with an appropriate control group were used. At 10 months of age, some animals underwent cCNDN (CND-cCNDN) while the remaining underwent sham procedure (CND-intact; control-intact). At 2 months post-cCNDN, BP, renal function and plasma renin activity (PRA) were assessed before, during and after hemorrhage (20% blood volume withdrawn over 15 minutes).

Results: Sheep with hypertension and CND that underwent cCNDN had similar BP to control sheep. Sheep that underwent cCNDN had significantly greater urinary excretion of sodium (control-cCNDN vs. control-intact; P=0.01; CND-cCNDN vs. CND-intact; P=0.04) compared to their intact counterparts. In response to hemorrhage, BP fell in all groups but the greatest decrease occurred in CND-cCNDN. In control-intact sheep this fall in BP gradually recovered, whereas in CND-cCNDN BP continued to rise. During hemorrhage, PRA continued to rise in CND-intact sheep while it decreased in CND-cCNDN sheep.

Conclusion: cCNDN appears to have adverse effects on BP, renal function and responses to hemorrhage in hypertensive sheep with CND.
Conclusions: cDNX effectively reduced BP at 2 months post-cDNX in previously hypertensive stroke. However, the lack of reflex activation of neural mechanisms observed during hemorrhage suggest that cDNX may impair a patient’s ability to adequately respond to physiological challenges.

THE POTENTIAL THERAPEUTIC VALUE OF ALLOPREGNANOLONE TO TARGET STRESS-RELATED HYPERTENSION

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Background: Stress-related hypertension is associated with increased activity and functional connectivity of the forebrain that integrate the response to stress. Hyperactive Schlager (BPH/2J) mice serve as a model for stress-related hypertension, displaying hyperactivity of stress pathways in addition to exaggerated pressor responses to stress. Functional alterations in the receptors of the major inhibitory mediator, GABA, are evident across numerous models of hypertension, including BPH/2J mice. Allopregnanolone is an endogenous neurosteroid reduced by chronic stress and which serves to enhance tonic inhibition through positive allosteric modulation of GABA receptors.

Aim: To determine whether allopregnanolone reduces both basal blood pressure and the pressor response to stress when administered centrally and peripherally.

Methods: Mice received allopregnanolone or its vehicle for a period of two weeks via two treatment paradigms, an intracerebroventricular cannula (0.31 mg/kg/day) or a subcutaneous minipump (0.31 mg/kg/day or 5 mg/kg/day). Prior implantation of telemetric probes enabled cardiovascular recordings before and after minipump insertion or attachment to the chronic guide cannula. The cardiovascular response to a diet cage switch and restraint were recorded before and after minipump implantation. Changes in GABA, mRNA expression were assessed by semi-quantitative reverse transcriptase (qRT)-PCR and changes in neuronal activity were assessed by Fos expression.

Results: Baseline and high dose peripheral delivery of allopregnanolone selectively reduced mean arterial pressure (7.2±2.9 mmHg, baseline; 12±6.3 mmHg, peripherally, P<0.04) and the pressor response to aminophylline (BPH/2J mice by 13±19%, P<0.003 for all). These effects were not observed for low dose peripheral delivery of allopregnanolone in BPH/2J mice or any of the treatment paradigms in BPH/Li mice. Allopregnanolone abolished the elevated Fos expression observed in BPH/2J mice in both the medial amygdala and hypothalamus. Additionally, the expression of hypothalamic GABA receptor subunits (α, δ and β2) mediating tonic inhibition were increased following allopregnanolone selectively in BPH/2J mice (P<0.005 for all).

Conclusion: Taken together the present results suggest that allopregnanolone mediates reductions in both mean arterial pressure and the pressor response to stress through central mechanisms and not peripheral mechanisms. As GABA dysfunction appears to be conserved across numerous models of hypertension, the findings highlight a potential clinical avenue for the treatment of stress-related hypertension.

BENEFICIAL CARDIAC EFFECTS OF THE ANGIOTENSIN CONVERTING ENZYME 2 ACTIVATOR DIMINAZENE IN AN EXPERIMENTAL MODEL OF KIDNEY DISEASE

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Background: The prevalence of kidney disease is increasing worldwide and cardiovascular disease remains the major cause of morbidity and mortality. Angiotensin converting enzyme (ACE) 2 is an important modulator of the renin-angiotensin system (RAS) through its role in hydrolysis of angiotensin (Ang) I. ACE2 can be activated by dimazene acetate (DIZE). Kidney disease secondary to subtotal nephrectomy (STNx) is associated with activation of the traditional RAS pathway, kidney impairment and adverse cardiac remodelling.

Aim: To assess the effects of short-term DIZE on cardiac structure and function in control and STNx rats.

Methods: Female Sprague Dawley rats underwent STNx and were treated for 2 weeks with either vehicle or DIZE (15 mg/kg/day, s.c.). Control rats were also treated with vehicle or DIZE (all groups, n=8 per group). Blood pressure and cardiac function were measured by catheterization of the left ventricle.

Results: STNx rats were hypertensive (BP difference: P<0.01) and exhibited cardiac hypercontractility and diastolic dysfunction (both P<0.05), left ventricular hypertrophy (P=0.001), interstitial (P=0.001) and perivascular (P=0.01) fibrosis, and elevated cardiac brain natriuretic peptide (BNP) mRNA (P=0.001). STNx rats had elevated cardiac ACE2 and ACE activity (both P<0.05 vs. control). In STNx, DIZE improved diastolic function, interstitial and perivascular fibrosis (all P<0.05 vs. STNx vehicle), and reduced BNP mRNA (P<0.05). The cardiac benefits of DIZE were associated with reduced cardiac ACE activity (P<0.05) and continued elevation in cardiac and plasma ACE2 activity.

Conclusion: DIZE shifted the cardiac ACE and ACE2 activity balance to a cardioprotective profile in STNx rats, but had no effect in control rats with normal kidney function and a balanced RAS. It remains unclear whether DIZE has direct effects to stimulate ACE2 activity or whether the effects in ACE2 activity is secondary to effects to maintain the increase in ACE2 mRNA abundance. Studies are needed to investigate if combining DIZE with standard RAS blockade has additive effects to ameliorate the adverse cardiac consequences of kidney disease.

AGE-DEPENDENT BLOOD PRESSURE DIFFERENCES OVER CONSECUTIVE MEASUREMENTS: IMPLICATIONS FOR HYPERTENSION DIAGNOSIS AND GUIDELINES

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Background: There is anecdotal belief that clinical 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 (aged 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 BP, 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

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Background: Abdominal aortic aneurysm (AAA) affects ~5% of men aged >65 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 the 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), 3.8 mg/mL (low dose), 15.4 mg/mL (medium dose) and 62.9 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. 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 infra-renal 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.

VASOULAR T CELL-ANTIGEN PRESENTING CELL INTERACTIONS DURING HYPERTENSION


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Background: T cells contribute to the development of experimental hypertension, which is associated with significant accumulation of T cells in the perivascular fat surrounding the aorta and renal vasculature. While a hypertension-specific neoantigen has been implicated in T cell activation, it is not known whether vascular-infiltrating T cells recognize, and are
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 (1.7 mg/kg/day for 14 days; nT cells) C5Bl/6J mice. Following anti-CD3/CD28 stimulation for 48 hours, cells were fluorescently labeled and co-incubated simultaneously for 16 hours with explanted aorta from normotensive or hypertensive C3H/101F2-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 C3H/101F2-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 nT cells compared to nT cells within Ang II-infused mouse aorta (390±113 vs. 208±66/mm²). Currently, time-lapse imaging systems showing that hypertensive mouse aorta showed that nT cells exhibited significantly slower velocity (nT cells 2.6 µm/min vs. 4.4 µm/min for nT cells; P<0.01; n=8–11), longer duration of interaction (nT 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 (nT cells 10.7±2.3% vs. 1.5±1.0% for nT cells; P<0.01; n=8–11). Direct activation of local vaso-occuls thereby giving rise to an expanded pool of monocytes. Splenic monocytes have been shown to accumulate in atherosclerotic plaques, and this was reduced with propranolol. However, unlike acute models of SNS-overdrive, extramedullary cell mobilization and monocytosis, thereby causing increased atherosclerosis. Aims: To determine the effects of fludrocortisone on NCC and pNCC, and thereby exclude primary aldosteronism. Urinary exosomes were isolated by progressive ultrafiltration and RCC, pNCC, WKYM and SPK expression were quantified by immunoblot, expressed as arbitrary units of optical density (OD)/mg creatinine to normalize for variation in urine concentration.

Results: We observed a progressive rise in NCC and pNCC abundance, with NCC increasing 3.1-fold (P<0.01) and median pNCC increasing 2.6-fold (P<0.01) during fludrocortisone administration. The ratio of pNCC/NCC, however, decreased by 49% (P<0.01). Abundance of WKYM increased by 3.3-fold (P<0.01) and SPK increased by 2.2-fold (P<0.01). Patients treated with adrenalectomy for aldosterone-producing adenoma (n=6) had lower baseline aldosterone levels (352 vs. 681 pmol/L; P=0.08) and higher renin levels (14 vs. 5.5 mU/L; P=0.036) and potassium levels (4.4 vs. 3.4 mmol/L; P=0.013) than pre-treatment, but baseline levels of NCC, WKYM, and SPK levels were not significantly different.

Conclusions: Mineralocorticoid administration causes a rapid and progressive increase in abundance and phosphorylation of NCC in humans. NCC abundance is stimulated to a greater extent than phosphorylation, and this appears to be via stimulation of the WKYM pathway, with a lesser effect on SPK. The findings widen the apparent role of aldosterone/mineralocorticoid receptor activation on distal renal tubular sodium handling and the importance of targeting this pathway for antihypertensive therapy.

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

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Background: Patients with chronic high blood pressure (BP) are at high risk of atherosclerotic cardiovascular disease. Because hypertension is a multifactorial disease, the exact mechanism(s) leading to the progression of atherosclerosis remain unknown. An important driving factor for atherosclerosis is sympathetic nervous system (SNS), involving noradrenaline (NE) binding to adrenoceptors. Sympathetic overdrive has been recently shown to repress the network for hematopoietic stem cells in the bone marrow (BM) inducing mobilization. In atherosclerosis and other inflammatory diseases, mobilized stem cells home to the spleen where extravascular hematopoiesis occurs. We hypothesize that sympathetic overdrive blocks this hematopoietic process and is SNS-driven.

Aims: To determine the effects of fludrocortisone on NCC, pNCC, WKYM and SPK in human urinary exosomes. To determine the effects of fludrocortisone on NCC and pNCC, and thereby exclude primary aldosteronism. Urinary exosomes were isolated by progressive ultrafiltration and RCC, pNCC, WKYM and SPK expression were quantified by immunoblot, expressed as arbitrary units of optical density (OD)/mg creatinine to normalize for variation in urine concentration.

Results: We observed a progressive rise in NCC and pNCC abundance, with NCC increasing 3.1-fold (P<0.01) and median pNCC increasing 2.6-fold (P<0.01) during fludrocortisone administration. The ratio of pNCC/NCC, however, decreased by 49% (P<0.01). Abundance of WKYM increased by 3.3-fold (P<0.01) and SPK increased by 2.2-fold (P<0.01). Patients treated with adrenalectomy for aldosterone-producing adenoma (n=6) had lower baseline aldosterone levels (352 vs. 681 pmol/L; P=0.08) and higher renin levels (14 vs. 5.5 mU/L; P=0.036) and potassium levels (4.4 vs. 3.4 mmol/L; P=0.013) than pre-treatment, but baseline levels of NCC, WKYM, and SPK levels were not significantly different.

Conclusions: Mineralocorticoid administration causes a rapid and progressive increase in abundance and phosphorylation of NCC in humans. NCC abundance is stimulated to a greater extent than phosphorylation, and this appears to be via stimulation of the WKYM pathway, with a lesser effect on SPK. The findings widen the apparent role of aldosterone/mineralocorticoid receptor activation on distal renal tubular sodium handling and the importance of targeting this pathway for antihypertensive therapy.

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

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Background: Nitric oxide (NO) is an important vascular signaling molecule that plays a role in the control of vascular function and the prevention of cardiovascular disease. The majority of NO is synthesized endogenously by endothelial nitric oxide synthase (eNOS). An alternate pathway involves exogenous dietary nitrate, which when converted to nitrate can be stored or used immediately. Atherosclerosis is associated with endothelial dysfunction and subsequent lesion formation. This is thought to arise from a reduction in the bioavailability and/or bioactivity of endogenous NO.

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

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Background: Placental growth factor (PLGF) is deficient in women with preeclampsia and treatment of experimental preeclampsia with parental PLGF has been successful in ameliorating features of preeclampsia in two different animal models. Abnormal placentation is thought to be the initiating event in the pathogenesis of preeclampsia. This process is mediated by angiogenic factors including PLGF.

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 (gd) 13 onwards. On gd 13, experimental preeclampsia was induced by 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), PLGF-2 were subjected to mult-i-stitch, écho-vascular 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 proteinuria (449±8.231 vs. 506±7.4 mg/mmol; P=0.59) between mice receiving control or PLGF-2. Serum FLT-1/PLGF ratio was significantly higher in mice administered PLGF-2 (333±18.6 vs. 493±44.6; P=0.007). There was no observed difference in T2 labyrinths/junctional zone ratio (P=0.59) in mouse placentas imaged (control, 2.2±0.01; n=18, vs. PLGF, 2.2±0.01 n=16).

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

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

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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 height²7: >115/95 g/m² or >49/45 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, 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 compared to the short-term (1.96; 1.11–3.45; P=0.02) and any first 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

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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 = 2.0–16.8 mmHg, non-diabetic range = 0.7–16.0 mmHg). In the meta-regression, duration of T2DM explained 16.3% 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: Increased AP and AIx, 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


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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 99 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±18 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 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


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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 prevention, the CVD absolute risk approach has been adopted in Australia. However, this approach has become a matter of concern among clinicians who hesitate at adopting a treatment threshold based on the absolute risk of an individual and that based on the traditional individual risk factor of blood pressure (BP). Updated guidelines do not routinely recommend BP lowering drug therapy in a low absolute CVD risk population (a risk of a CVD event of less than 10% in the next 5 years) unless a systolic BP threshold of 160 mmHg is exceeded. Many GPs have expressed a concern that delaying pharmacotherapy may lead to irreversible target organ damage, a so called “legacy effect.” It is therefore timely to conduct a study addressing the question of whether earlier active BP lowering pharmacotherapy brings therapeutic benefits for a low risk population over their lifetime.

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 will 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 late 1980s on 3,427 participants aged 40–69 years who were free from the general population with mildly elevated BP and no history of CVD or diabetes. We plan to probability match all participants to the Australia Institute of Health and Welfare National Death Index,
and classify the cause of death by the international Classification of Disease version 10. All analyses will be based on the “intention to treat” principle. Cox proportional hazard models will be used to estimate hazard ratios and corresponding 95% confidence intervals.

Results: To date we have retrieved ANBP study archives and received funding from the Royal Australian College of General Practitioners Research Foundation. An ethics application is being prepared. Interim results will be presented if available.

Conclusion: The present findings might contribute to increasing the adoption of current guidelines into 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 HYPERTENSIVE BPH/2J MICE

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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 mice and normotensive BPN/3J control mice (n=6–10) were administered miR/vana miR-181a mimic or vehicle as negative control (0, 1, 5, 1, and 25 mmol, i.v) using an in vivo kidney-specific transfection reagent (Altoogen). Blood pressure (BP) was measured in urethane and for two days following treatment via pre-implanted radiotelemetry probes. The BP response to angiotensin converting enzyme (ACE) inhibition (enalaprilat) and ganglion blockade (pentolinium) was determined during the dark period (~26 h after a 25 mmol dose and kidney tissue was collected at ~50 hours for measurement of renin mRNA.

Results: The peak hypotensive effect of the mimic relative to vehicle treatment in BPH/2J mice was observed 12–15 h after the 5 mmol dose (~5.8±1.5 mmHg), which was greater than the effect in BPH/2J mice treated with the negative control (0.7±1.0 mmHg; P<0.02). However, the effect of the 1 and 25 mmol doses of mimic on BP were comparable between strains and with the negative control (vehicle treatment; P>0.12). Renal renin mRNA abundance in BPH/2J mice treated with the miR-181a mimic was lower than BPH/2J mice treated with the negative control (3.5±0.6 vs. 4.8±1.1; P>0.01), suggesting that the mimic effectively inhibited renin mRNA in vivo. By contrast renin mRNA was comparable in BPH/2J mice treated with either the mimic or negative control, respectively (3.1±0.7 vs. 2.6±0.7; P>0.45). Furthermore the depressor response to enalaprilat in BPH/2J mice treated with the negative control was abolished in BPH/2J mice treated with the mimic (~17±3 mmHg vs. ~1±3 mmHg; respectively, P<0.001), suggesting the mimic reduced the RAS contribution to BP maintenance. The depressor response to pentolinium following enalaprilat pre-treatment was comparable between negative control and mimic-treated BPH/2J mice (~5±2.5 vs. ~5±1.3 mmHg; P>0.80), suggesting the mimic does not overtly affect the SNS contribution to BP in BPH/2J mice.

Conclusion: The present findings provide the first in vivo evidence that low miR-181a levels contribute to greater renal renin mRNA level and thereby a contribution of the RAS to the hypertension in BPH/2J mice.

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

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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 overweight postmenopausal women (BMI 28.6±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 sprinting on a cycle ergometer followed by 12 s of easy pedalling, repeated for a total of 20 minutes. 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 sprinting on a cycle ergometer followed by 12 s of easy pedalling, repeated for a total of 20 minutes.

Results: ISE compared to control women significantly (P<0.05) improved their aerobic fitness (2.33±0.11 vs. 1.79±0.11 L/min). Baroreceptor sensitivity of the ISE (9.35±0.81 ms/mmHg) increased significantly at post-test (P<0.05) compared to the control group (6.86±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 POLYGENIC MODEL OF CARDIAC HYPERTROPHY

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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 polygenic forms of the disease.

Aim: To use the hypertrophic heart rat (HHR), a unique normotensive polygenic 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 (7th) was differentially expressed in all age groups (FDR<0.1; P<0.05). 7th is involved in cardiac amyloidosis, infiltrating cardiovascular structures, leading to hypertrophy. In rats older than 13 weeks old, we found expression of 4 genes (Actb, Ank1, 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, Ank1 (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 polygenic forms of the disease and thus merit further investigation.

BARORECEPTOR SENSITIVITY IN DIABETIC RATS WITH TREATED AND UntREATED HYPERTENSION

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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 through 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 diabetes (streptozotocin) and 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 minimum 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 mmHg; P=0.95) or diabetic rats (1.25±0.29 mmHg vs. diabetes+Tx 1.46±0.14 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 MDIVI-1 IMPROVES SURVIVAL OF HUMAN CARDIAC RESIDENT STEM CELLS

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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.

**Aim:** To determine whether Mdivi-1, an inhibitor of mitochondrial fission protein DRP1, can improve survival of a novel population of human CRSCs.

**Methods:** Mdivi-1 significantly reduced 4,0'-induced cell death at 50 µM and 100 µM (P<0.05 vs. vehicle; n=8). 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 recovery 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 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.

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

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**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.

**Aim:** 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-trial-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 1.31–3.77; P=0.01), impaired renal function (AOR=2.03, 95% CI 1.07–3.98; 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 diabetic BP (AOR=0.99, 95% CI 0.96–1.02; P=0.63), 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.

**Conclusion:** 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**

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**Background:** Blood pressure (BP) variability in children is not a routine clinical assessment, partly due to concerns about diagnostic accuracy and controversy over normative reference 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.

**Aim:** To determine the prevalence of hypertension and the role of BPV among the largest population study of Australian children to date.

**Methods:** Two consecutive BP measurements were recorded by oscillography in 3,047 children (aged 12 years; 95% CI 12.1, 12.3; males 52%) from the 2011–2013 Australian Health Survey. A 3rd BP reading was taken if the difference between the first and second BP readings (ΔSBP) was ≥10 mmHg. BPV was calculated as the coefficient of variation (CV; [SD/mean SBP] × 100) for children with three SBP readings.

**Results:** The prevalence of hypertension, as defined according to age, sex and height referenced 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 correlations of SBP were sex (β=4.1; P=0.03), height (β=0.19; P=0.001), body mass index (BMI; β=0.61; P=0.001), serum vitamin B12 (β=0.07; P=0.005), serum ferritin (β=0.01; P=0.018) and urinary 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]; 7.8 mmHg [95% CI 5.7, 9.3]; P=0.004 and ASSBP: 13.9 mmHg [95% CI 9.5, 18.4] vs. 6.4 mmHg [95% CI 6.0, 6.7]; P=0.001). Minimum ≤0.25 corresponding to normal BP ranges irrespective of age.

**Conclusions:** Hypertension prevalence is 4% among Australian children, and is associated with increased BPV, 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**


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**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 Schlager mice. Changes in intrarenal nerves in diabetes have not, however, been investigated previously.

**Aim:** To investigate the effect of diabetes on neural staining and catecholamine content in kidneys of Schlager mice with (BPH/2J) and without (BPN/3J) concomitant hypertension.

**Methods:** After 10 weeks of study, hypertensive BPH/2J and normotensive BPH/2J Schlager 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.
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

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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 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 laterализation 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 (P=0.12).

Overall 38 procedures achieved adequate cortisol gradients both pre and post-ACTH, and in 33 of these a clear diagnostic indication of unilateral or bilateral was achieved both pre and post-ACTH. 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


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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/headache, 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 <5), and 9 (26%) had mild (AHI 5–15), 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=0.34; 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.