

## AMBULATORY BLOOD PRESSURE MONITORING MAY FLAG PRIMARY ALDOSTERONISM IN HYPERTENSIVE PATIENTS

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**Background:** Primary aldosteronism (PA) has a reported prevalence of up to 20% in cases of resistant hypertension, but is substantially under-diagnosed due to the lack of specific symptoms and signs. Ambulatory blood pressure (AMBP) monitoring provides a non-invasive method for evaluating circadian BP variations, offers valuable prognostic information and may help to identify PA in patients referred with non-specific hypertension for investigation.

**Aims:** To compare AMBP parameters in hypertensive patients with established PA and those without, and correlate these parameters with cardiovascular outcomes.

**Methods:** AMBP readings were evaluated retrospectively in 407 patients assessed at Monash Heart. Patient demographics, screening aldosterone and renin concentrations, as well as medications were retrieved from medical records. We identified 396 non-PA and 11 PA patients and their cardiovascular events (myocardial infarction, left ventricular hypertrophy, coronary artery disease, atrial fibrillation) were recorded. Statistical significance was set at  $P < 0.05$ .

**Results:** Compared to hypertensive patients without established PA, PA patients were younger (mean:  $51.5 \pm 13.3$  vs.  $62.2 \pm 14.2$  years), had higher BP readings (mean:  $150/86 \pm 20.5/7.4$  vs.  $134/75 \pm 17.2/10.7$  mmHg) with similar patterns observed for average daytime and night-time BP. BP load (% daytime and night-time SBP/DBP readings over 135/85 and 120/70 mmHg, respectively) was significantly higher for both systolic and diastolic in PA (mean:  $72.4 \pm 26.4$  and  $50.2 \pm 25.6$  %) compared with the non-PA group (mean:  $49.3 \pm 28.5$  and  $21.6 \pm 22.7$  %). We found that 81% of patients with PA (9/11) had loss of physiological nocturnal BP dipping compared with 44% of the non-PA group (175/396). Rates of cardiovascular events were similar in both groups but may have been confounded by the retrospective nature of this study and lack of long-term follow-up.

**Conclusion:** Our study found that PA was associated with a significant increase in BP load and loss of nocturnal BP dipping which are known risk factors for adverse cardiovascular events. A prospective study is needed to better define AMBP parameters in PA and evaluate their ability to flag underlying PA amongst hypertensive patients.

## MEASURING BLOOD PRESSURE DEPENDENCY OF LARGE ARTERY STIFFNESS USING POSTURAL CHANGE

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**Background:** Large artery stiffness, often measured by carotid-femoral pulse wave velocity (cfPWV) is a cardiovascular risk factor, but threshold values are made difficult due to the dependency on the distending blood pressure (BP).

**Aims:** To measure BP dependency of cfPWV in individuals in a clinically approachable manner as a method of addressing the interaction between BP and arterial stiffness.

**Methods:** Brachial BP (oscillometric monitor) and cfPWV (simultaneous carotid tonometry and femoral cuff) were measured in the seated and supine position. The postural change can invoke a change in systemic BP, but will always cause a significant change in hydrostatic pressure across the carotid-femoral path-length. The hydrostatic effect was calculated using vascular path-length estimated from body surface measurements. Change in cfPWV from seated to supine was divided by the combined change in systemic diastolic BP and hydrostatic pressure ( $\Delta\text{PWV}/\Delta\text{DP}$ ), the BP dependency of cfPWV. Stepwise linear regression was used to investigate demographic and cardiovascular correlates.

**Results:** We recruited 88 subjects (19–91 years, 41 female). Net BP decreased in all subjects with change in posture from seated to supine and cfPWV decreased in all but 4 subjects. Average  $\Delta\text{PWV}/\Delta\text{DP}$  was  $6\pm 3$  cm/s/mmHg (range 0.9 to 10.5 cm/s/mmHg) and correlated with seated brachial pulse pressure ( $\beta=0.40$ ,  $P<0.001$ ), diastolic pressure ( $\beta=-0.33$ ,  $P<0.001$ ), gender ( $\beta=0.25$ ,  $P=0.010$ ), and heart rate ( $\beta=0.23$ ,  $P=0.033$ ) with no correlation with supine cfPWV or age.

**Conclusion:** Measuring BP and cfPWV seated and supine provides a clinically feasible approach for measuring the BP dependency of large artery stiffness. Whilst this study provides some correlative results, larger studies are required to investigate the dependency of the parameter, and ultimately if it provides a better estimation of cardiovascular risk than cfPWV alone.

## THE GLOBAL SYMPLICITY REGISTRY: SAFETY AND EFFICACY REPORT

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**Background:** The aim of the Global SYMPLICITY Registry (GSR) is to collect real-world data on the safety and efficacy of renal denervation (RDN) using either the original Symplicity Flex™ renal denervation catheter or the newer-generation Symplicity Spyral™ catheter. Furthermore, a sub-cohort of patients are receiving treatment of renal artery branch vessels in addition to the main renal artery.

**Aims:** To determine safety and efficacy of the Simplicity renal denervation system.

**Methods:** The GSR is a prospective, multi-centre, non-randomized international registry of RDN enrolling up to 3000 patients with uncontrolled hypertension. Patients are followed at 3, 6, 12, 24 and 36 months. Follow-up data collected per routine care includes: clinical assessment, office blood pressure measurement, 24-hour ambulatory blood pressure measurement, blood tests, ECGs, renal artery imaging, and EQ-5D quality of life questionnaire. At the time of the HBPRCA 2017 meeting, six month safety and efficacy data will be available for ~2500 patients and 3-year data will be available on ~1750 patients. Moreover, data from post-hoc analysis of ~270 patients treated with the Symplicity Spyral catheter as well as data on ~90 patients who had RDN treatment in both the main renal artery and branches will be available for presentation.

**Results and Conclusions:** The Global SYMPLICITY Registry is the largest real world database of renal denervation therapy and has enrolled over 2500 patients to date. Thus far, no long-term safety concerns following the renal denervation procedure have been observed with the Symplicity Flex™ or Spyral™ systems. Renal denervation in this large real world population resulted in significant reductions in both office and ambulatory blood pressure that were sustained out to 3 years post-procedure. All available follow-up data informing on short and long-term safety and efficacy of the Symplicity renal denervation system will be presented.

## NOVEL IDENTIFICATION OF NOCTURNAL HYPERTENSION AND THE PREDICTION OF CARDIOVASCULAR DEATH IN A GENERAL POPULATION

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**Background:** Subjects with nocturnal hypertension often show a non-dipping pattern and have higher risk of cardiovascular mortality. Usually, time-based classification of nocturnal dipping is used to define risks but this is limited by the variability of dipping patterns amongst subjects (e.g., early risers and late risers).

**Aim:** To apply a newly developed method to classify non-dipping using a 6 parameter logistic equation to determine the exact magnitude of the dip irrespective of time, where we defined non-dipping as the range of mean blood pressure (BP) between the upper and lower plateaus.

**Methods:** We examined the prognostic value of a <10% reduction in range versus the conventional day-night difference of <10% using data from the Ohasama 15-year outcome study.

**Results:** Among 1535 subjects, 7% (n=110) were categorized as non-dippers by our new range classification (R-ND). They had higher nocturnal mean BP (89±10 vs. 83±9 mmHg;  $P < 0.01$ ), lower day-time mean BP (89±10 vs. 93±10 mmHg;  $P < 0.01$ ), similar 24h average mean BP (88±10 vs. 89±9 mmHg), and were older (66±11 vs. 61±11,  $P < 0.01$ ), compared with dippers. The conventional classification yielded 54% (n=823) defined as non-dippers at night (C-ND). Of these, 723 subjects dipped early or late but not at night. The C-ND group had higher nocturnal mean BP (87±10 vs. 80±7 mmHg;  $P < 0.01$ ), lower day-time mean BP (90±10 vs. 95±9 mmHg;  $P < 0.01$ ), similar 24h average mean BP (89±9 vs 89±8 mmHg), and were older (64±11 vs 60±10;  $P < 0.01$ ), compared with dippers. Both R-ND and C-ND were associated with similar adverse cardiovascular events (cardiovascular death odds ratio 1.8 and 2.3, respectively,  $P < 0.002$ ), but only C-ND predicted non-fatal stroke (odds ratio 1.6,  $P = 0.005$ ; for R-ND 1.2,  $P = 0.5$ ). After adjustment for age, R-ND and C-ND predicted all cause death equally (odds ratios 1.7 and 1.6;  $P < 0.03$ ).

**Conclusion:** Our novel method of defining non-dippers selected a very small group of only 7% compared to the traditional method, but did not improve the calculated risk of cardiovascular events. These findings suggest that dipping early or late indicate equal risk of death compared to subjects not dipping at night.

## TEN-YEAR LEGACY EFFECTS OF DELAYED LIPID LOWERING DRUG TREATMENT ON CARDIOVASCULAR DISEASE IN THE SECOND AUSTRALIAN NATIONAL BLOOD PRESSURE STUDY (ANBP2)

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**Background:** Lipid lowering drug treatment (LLT) provides benefits for the primary prevention of cardiovascular disease (CVD) in high risk populations. The benefits may vary considerably depending on the initial levels of CVD risk, irrespective of initial lipid levels. There is evidence supporting the benefits of LLT for sufficiently high CVD risk populations, but limited evidence for lower risk populations, and mixed evidence for the elderly.

**Aims:** To investigate the effects of LLT in the elderly who had baseline LLT (early treatment) and those who did not (delayed treatment) on all-cause and CVD mortality in a randomized trial of BP-lowering therapy.

**Methods:** We conducted a post-hoc survival analysis of participants in the Second Australian National Blood Pressure study (ANBP2). ANBP2 was a randomized, open-label study with blinded end point assessment in participants aged 65 to 84 years. We re-stratified the participants into two groups: those who were undergoing LLT at trial entry and those who were not, regardless of randomization status within the trial. We also excluded participants who had a previous history of CVD and those who initiated LLT during the trial period. We calculated 4-year in-trial fatal and non-fatal outcomes, and extended 6-year post-trial fatal outcomes. Thus a 10-year legacy effect of delayed LLT could be tested.

**Results:** The analysis included 4257 participants (LLT group: 648, non-LLT group: 3609) aged 72.0 ± 5.0 years who at baseline had an average 5-year CVD risk (18.3 ± 7.5%) with a total cholesterol level of 5.5 ± 0.9 mmol/l. In the overall study population, we found a significant reduction in all-cause mortality (HR 0.65, 95% CI 0.43–0.98; *P* = 0.04) for the group treated with LLT, but also a statistically significant increase in new onset diabetes observed (HR 1.51, 95% CI 1.05–2.16; *P* = 0.03) during the in-trial period. Over a 10-year follow-up period (including post-trial), the effects became significant for cancer death with a HR of 0.55 (95%CI 0.39–0.78; *P* = 0.001) and all-cause mortality remained significant with a HR of 0.76 (95% CI 0.63–0.91; *P*=0.003). No significant effects were found for CVD mortality. All analyses were adjusted for baseline characteristics.

**Conclusion:** Our study showed legacy effects of delayed LLT regarding deaths from any causes and cancer. Thus, our finding contributes evidence to recommend the earlier treatment of LLT in high CVD risk older individuals (age > 65 years).

## COMPARISON OF CENTRAL AND PERIPHERAL ARTERIAL PRESSURE FOR ASSESSMENT OF SUBCLINICAL TARGET ORGAN DAMAGE

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**Background:** Markers of vascular function and incidence of cardiovascular events have been shown to be more strongly associated with central aortic pressure (cAP) compared to peripheral arterial pressure (pAP). However, the potential clinical use of hemodynamic indices of cAP and pAP as markers of target organ damage (TOD) has not been well established.

**Aims:** To assess the association of cAP and pAP with markers of TOD and to seek any differences related to age.

**Methods:** Conventional indices of cAP and pAP pulse waveforms (pulse pressure [aPP, pPP], waveshape form factor and augmentation index) were assessed in relation to TOD in 770 hospital inpatients (age 60.0±10.0 years, 473 males) with primary hypertension. TOD was quantified in terms of arterial stiffness as measured by carotid-femoral pulse wave velocity (cfPWV), carotid intima-media thickness (IMT) and urine albumin-to-creatinine ratio (ACR). Subclinical TOD was defined as carotid IMT > 0.9 mm, urine ACR > 3.5 mg/mmol in females and > 2.5 mg/mmol in males and/or cfPWV > 12 m/s.

**Results:** For the whole cohort, cPP and pPP showed a significant positive correlation with cfPWV ( $r = 0.41$  vs.  $r = 0.40$ ;  $P < 0.01$ ), ACR ( $r = 0.24$  vs.  $r = 0.27$ ;  $P < 0.01$ ) and carotid IMT ( $r = 0.14$  vs.  $r = 0.15$ ;  $P < 0.01$ ). Each SD increase in pPP and cPP was associated with respective odds ratios (OR) of 2.7, 2.9 (cfPWV), 1.2, 1.4 (ACR), 1.46, 1.53 (IMT). When corrected for confounding variables, cPP had higher predictive power for TOD for age  $\geq 60$  years compared to pPP.

**Conclusion:** Both pPP and cP showed an association with TOD in a hypertensive population. However, compared to pPP, cPP provides independent and additional information associated with TOD in elderly hypertensive subjects. Additional hemodynamic indices of cAP as potential biomarkers of subclinical TOD need to be validated by further prospective studies.

## COMPARISON BETWEEN THE ALDOSTERONE/ RENIN RATIO AND THE ALDOSTERONE/ ANGIOTENSIN II RATIO AS A SCREENING TEST FOR PRIMARY ALDOSTERONISM

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**Background:** The upright plasma aldosterone/renin ratio (ARR) is widely used to screen for primary aldosteronism (PA). Many medications (including most antihypertensives) and various physiological factors can affect renin (more markedly) and aldosterone levels, resulting in false negative or positive ARR results. The ARR also appears mathematically more dependent on renin than aldosterone, especially when renin levels are low (as in patients with PA), small absolute changes of renin can result in large changes in the ARR. The aldosterone/angiotensin II ratio (AA2R), with quantification of angiotensin II (the main direct regulator of aldosterone biosynthesis) rather than its upstream renin (has no direct effect on aldosterone secretion), may have the potential for superior case detection of PA.

**Aims:** To compare the performance of the AA2R with the ARR in PA screening.

**Methods:** Bloods were collected at midmorning in an upright position from 48 patients with PA and 20 patients without PA. Serum aldosterone concentration (Aldo) was measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Direct renin concentration (DRC) was determined by chemiluminescent immunoassay. Serum equilibrium angiotensin II (eqAngII) was measured using a novel renin-angiotensin system (RAS) equilibrium analysis based on LC-MS/MS. The ARR and the AA2R were calculated and the areas under the ROC curves were compared.

**Results:** PA patients displayed significantly ( $P < 0.01$ ) higher levels of Aldo, the ARR, and the AA2R than non-PA patients, and significantly ( $P < 0.01$ ) lower levels of DRC, eqAngII, and  $K^+$ , as expected. The area under the ROC curve (AUC) of the upright ARR was 0.932 (95% confidence interval (CI): 0.844-0.979), while the AUC for the upright AA2R was 0.916 (0.823–0.969). The AUC for the two ratios were both significantly ( $P < 0.01$ ) larger than the area under the reference line (AUC=0.5), but were not significantly different ( $P > 0.05$ ). The calculated diagnostic cut-off value of the upright ARR [(pmol/L)/(mU/L)] was 53 (Youden Index (YI) = 0.81, sensitivity=0.96, Specificity=0.85), which is very close to the value we use clinically (at 55 y), while the calculated cut-off value of the upright AA2R (pmol/L)/(pmol/L) was 12 (YI=0.75, sensitivity=0.85, specificity=0.90). Using 53 and 12 as the threshold, the incidences of false positive and false negative ARR in this study were 15% and 4.2%, while the incidences of false positive and false negative AA2R were 10% and 14.6%, respectively.

**Conclusion:** In this pilot study, the AA2R appeared to perform at least equivalently to the ARR in screening for PA. Further studies are planned to validate this AA2R among a larger population and to compare its performance with the ARR during treatment with various antihypertensive medications.

## CAN EFFECTS OF MINERALOCORTICOIDS ON ENDOTHELIAL SODIUM CHANNEL GAMMA-SUBUNIT CLEAVAGE IN HUMAN BE ASSESSED NON-INVASIVELY BY STUDYING URINARY EXOSOMES?

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**Background:** Primary aldosteronism (PA), a common and potentially curable form of hypertension, is characterized by excessive and autonomous production of aldosterone by the adrenal glands. Aldosterone is the major regulator of sodium and potassium excretion due to its effects on the epithelial sodium channel (ENaC) and sodium chloride cotransporter (NCC) in the renal distal nephron through various mechanisms. Relevant animal studies demonstrate aldosterone stimulates prostasin to activate ENaC by increasing  $\alpha$ -subunit ( $\alpha$ -ENaC) abundance and inducing  $\gamma$ -subunit ( $\gamma$ -ENaC) cleavage. Urinary exosome extraction techniques enable the study of sodium transporters non-invasively in humans.

**Aims:** To explore urinary exosomal  $\gamma$ -ENaC abundance in hypertensive patients undergoing fludrocortisone (mineralocorticoid) suppression testing (FST) to diagnose or exclude PA, as a means of assessing  $\gamma$ -ENaC cleavage induced by mineralocorticoid administration in humans.

**Methods:** Morning spot urine samples from hypertensive patients (n=13, 6 females/7 males) who underwent FST were collected basally (D0) and after 4 days mineralocorticoid administration (D4), treated with protease inhibitor and were stored at  $-80^{\circ}\text{C}$ . Urinary exosomes were harvested by progressive ultracentrifugation, followed by immunoblotting to measure abundance of prostasin and cleaved forms of  $\gamma$ -ENaC.

**Results:** Preliminary data have demonstrated a trend towards an increase in prostasin in response to fludrocortisone administration (fold change = 1.26,  $P=0.07$ ). However, dominant  $\gamma$ -ENaC cleavage products (55 kDa) remained unchanged.

**Conclusion:** Prostasin and  $\gamma$ -ENaC cleavage are detectable in human urinary exosomes in PA and healthy subjects. A trend towards an increase in prostasin during 4-day mineralocorticoid administration was observed. Dominant  $\gamma$ -ENaC cleavage products (55 kDa) represent cleavage by prostasin/kallikrein, and remained unchanged (fold change = 1.05) in response to mineralocorticoid administration, which suggests that mineralocorticoid-induced increases in prostasin may not alter the abundance of prostasin/kallikrein  $\gamma$ -ENaC cleavage products in urinary exosomes.



## APPLICATION OF A NOVEL ANGIOTENSIN II ASSAY IN CONFIRMATORY TESTING FOR PRIMARY ALDOSTERONISM

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**Background:** Because the aldosterone/renin ratio (ARR) can be falsely elevated (false-positive) during case detection for primary aldosteronism (PA), confirmatory testing such as by the fludrocortisone suppression test (FST) is necessary to definitively confirm or exclude the diagnosis. Fludrocortisone, a synthetic steroid, possesses very potent mineralocorticoid properties along with comparatively very weak glucocorticoid activity. The basis of FST involves the demonstration of ongoing (non-suppressible) aldosterone production in the face of suppression of its main regulator, the renin-angiotensin system (RAS). Angiotensin II can now be measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS) and aldosterone/angiotensin II ratio (AA2R) can be calculated.

**Aims:** To validate a novel angiotensin II assay by evaluating the effects of FST on aldosterone, renin, and angiotensin II among PA and non-PA patients.

**Methods:** We administered 48 PA patients and 20 non-PA patients with 0.1 mg oral fludrocortisone Q6h for 4 days. Blood samples were collected at 7 AM following overnight recumbency and again at 10 AM in an upright position basally (day 0) and on day 4 of FST. PA was confirmed (FST-positive) if upright (at 10 AM) aldosterone was > 130 pmol/L on day 4 of FST, provided upright renin was < 8.4 mU/L, upright cortisol level was lower than recumbent (at 7 AM), and K<sup>+</sup> was in the normal range. Serum aldosterone concentration (Aldo) was measured by LC-MS/MS. Direct renin concentration (DRC) was determined by chemiluminescent immunoassay. Serum equilibrium angiotensin II (eqAngII) was measured using a novel RAS equilibrium analysis based on LC-MS/MS.

**Results:** Compared to day 0, both the PA and non-PA groups showed significant ( $P < 0.01$ ) decreases in Aldo, DRC, and eqAngII on day 4 of FST, but the decrease of Aldo at 7 AM in the non-PA group was not statistically significant ( $P > 0.05$ ). Although Aldo at 10 AM in PA patients fell from 423 (263–588) to 315 (192–476) pmol/L, values were still higher than 130 pmol/L in all patients (but in none of the non-PA patients), meeting the diagnostic criterion of confirming PA. On day 4 of FST, PA patients continued to show significantly ( $P < 0.01$ ) higher levels of Aldo, the ARR and the AA2R than non-PA patients, but the differences in DRC and eqAngII between the two groups were no longer significant ( $P > 0.05$ ) as both were suppressed to extremely low levels by fludrocortisone administration. DRC and eqAngII at 10 AM on day 4 of FST also showed significant ( $P < 0.01$ ) positive correlations in the two groups with Spearman correlation coefficients of 0.746 and 0.551, respectively.

**Conclusion:** The changes of renin and eqAngII, the ARR and AA2R all showed good consistency during FST, indicating that this novel angiotensin II assay may have the potential for future application in distinguishing PA and non-PA.

## CURRENT PATTERN OF PRIMARY ALDOSTERONISM DIAGNOSIS: DELAYED AND COMPLICATED

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**Background:** Primary aldosteronism (PA), also known as Conn's syndrome, is the most common specifically treatable and potentially curable cause of hypertension. It has a prevalence of 4.6-13% in patients with hypertension and up to 20% in patients with resistant hypertension, based primarily on overseas literature. There is limited data regarding the epidemiology and diagnosis of PA in Australia.

**Aims:** To analyse the referral pattern and disease characteristics of hypertensive patients referred to the Endocrine Hypertension Service at Monash Health.

**Methods:** We conducted a retrospective review of 99 patients who attended the Endocrine Hypertension Service since its establishment in May 2016. Each patient completed a questionnaire that covered socio-demographics, diet and exercise, diagnosis of hypertension, medications and comorbidities. Sources of referral, comorbidities, clinical outcomes, and biochemical outcomes were obtained from Scanned Medical Records and Monash Pathology database.

**Results:** Only 3% of referrals were made at the first presentation of hypertension, whilst 97% were made for complex hypertension and/or end organ damage. The majority of referrals (67%) was derived from tertiary centres while only 20% came from primary care centres. The diagnosis of PA was delayed, with the majority of patients having had hypertension for at least 6 years prior to their referral. On presentation, 32% of patients had chronic renal failure, left ventricular hypertrophy, stroke or atrial fibrillation. Of the patients diagnosed with PA, targeted management of aldosterone-producing adenoma (APA) by adrenalectomy resulted in complete resolution of hypertension in 75% and biochemical cure in 100%. Targeted management of bilateral adrenal hyperplasia or APA (where patient factors led to preferred medical management over adrenalectomy) also led to significant improvement in blood pressure control. All patients who underwent adrenalectomy and 73% of patients who received medical management required a decreased number of anti-hypertensive medications.

**Conclusion:** The diagnosis of PA remains delayed and complicated. The majority of patients are referred by tertiary institutions after enduring longstanding hypertension that is already complicated by end-organ damage. Given that appropriate management of PA resulted in significant clinical and biochemical improvement, we need to increase awareness of PA in primary care so that the diagnosis can be made early for maximal health benefits.

## ACUTE SODIUM LOADING DECREASES ABUNDANCE OF SODIUM CHLORIDE TRANSPORTER AND ITS PHOSPHORYLATION IN HUMAN URINARY EXOSOMES

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**Background:** Primary aldosteronism (PA), a common and potentially curable form of hypertension is characterized by excessive and autonomous production of aldosterone by the adrenal glands. Aldosterone serves as a signal to increase the expression and activity of sodium chloride cotransporter (NCC) and the epithelial sodium channel (ENaC) in response to a low sodium diet. Potassium is now also thought to be a major regulator of NCC. Acute sodium loading results in a rapid decrease of aldosterone in humans, and a concomitant inhibition of ENaC has been observed in murine studies.

**Aims:** To explore urinary exosomal NCC abundance and phosphorylation in hypertensive patients undergoing saline suppression testing (SST) to diagnose or exclude PA, as a means of non-invasively assessing variations of NCC abundance and its phosphorylation.

**Methods:** Urine samples collected from hypertensive patients who underwent SST (seated posture n=5 (3 female and 2 male), recumbent posture n=4 (1 female and 3 male)) were collected before and after 4-hour acute sodium loading. Samples were treated with protease inhibitor and stored at -80°C. Urinary exosomes were harvested by progressive ultracentrifugation, followed by immunoblotting to measure abundance of NCC and its phosphorylated form (pNCC).

**Results:** Preliminary data demonstrated a trend toward decrease in abundance of NCC (mean fold-change = 0.48) and a significant decrease in abundance of pNCC (mean fold-change = 0.48;  $P=0.029$ ) in response to 4-hour sodium loading in both seated and recumbent postures. The reduction in abundance of pNCC appeared to be greater in the seated posture (mean fold change = 0.34) compared to the recumbent posture (0.94). During 4-hour sodium loading, plasma potassium concentration was 3.95 mmol/L pre infusion and 3.94 mmol/L post infusion ( $P=0.91$ ), and plasma aldosterone was 256 pmol/L pre infusion and 121 pmol/L post infusion ( $P=0.001$ ) in both postures.

**Conclusion:** Changes in NCC abundance and its phosphorylation in response to acute sodium loading are detectable in human urinary exosomes in PA. The observable decrease in NCC abundance and significant decrease in pNCC abundance suggests acute sodium loading may at least partially induce sodium excretion by reducing NCC abundance and its phosphorylation, independent of plasma potassium. The apparent differences in findings between seated and recumbent postures requires further investigation.

## LONG-TERM EFFECT OF TRANSVENOUS CAROTID BODY ABLATION IN THE TREATMENT OF PATIENTS WITH RESISTANT HYPERTENSION

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**Background:** The carotid body (CB) is considered a therapeutic target in diseases mediated by the sympathetic nervous system.

**Aims:** To assess the safety and effectiveness of a catheter-based system to ablate the carotid body and reduce blood pressure (BP) in patients with resistant hypertension.

**Methods:** This single-arm multicenter prospective study was approved for treatment of 45 patients. Inclusion criteria included stability on three or more antihypertensive medications and daytime systolic ambulatory BP  $\geq 135$  mmHg. The primary safety endpoint was the rate of death, hospitalization for hypertensive crisis, and device- or procedure-related serious adverse event (SAE) at 1 month. The secondary safety endpoint was the rate of death and hospitalization for hypertensive crisis at 6, 12, 18 and 24 months. Effectiveness was defined as the change in 24-h systolic ambulatory BP between baseline and 1, 3, 6 and 12 months. The procedure was performed using a proprietary ablation catheter delivering ultrasound energy via the jugular vein with intravascular imaging guidance.

**Results:** To date, long-term follow up has been obtained in 22 patients. The study population consisted of patients with a mean age of 63 years, 69% male, mean BMI of  $30 \pm 4$  kg/m<sup>2</sup>, and diabetes in 22%. Mean baseline 24-h ABPM was  $154 \pm 13/94 \pm 12$  mmHg. Two instances of post-procedural chest pain have been reported to date that were resolved by the one-month follow up. One serious adverse event was reported related to groin access complications. One TIA was reported several hours after the procedure, likely related to procedural hypertension. The 24-h ABPM was reduced by an average of  $9 \pm 11/5 \pm 7$  mmHg at 1 month,  $8 \pm 16/5 \pm 10$  mmHg at 3 months, and  $11 \pm 13/7 \pm 8$  mmHg at 6 months.

**Conclusion:** Transvenous catheter-based ablation of the carotid body seems to be a promising approach, with results that persist over time. The results need to be confirmed in larger, randomized evaluations.

## N-ACETYLCYSTEINE REVERSES ESTABLISHED RENAL FIBROSIS AND RESTORES RENAL FUNCTION IN A MOUSE MODEL OF DILATED CARDIOMYOPATHY

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**Background:** Renal dysfunction occurring secondary to heart failure (HF) involves a complex interplay of mechanisms, among which renal fibrosis is a key culprit. It has been shown that levels of glutathione, the body's predominant antioxidant, when augmented contributes to fibrosis development in cardiac and renal dysfunction.

**Aims:** To determine if restoring glutathione capacity can reverse renal fibrosis and improve renal function in mice with HF.

**Methods:** Eighteen-week old mice with dilated cardiomyopathy (DCM; n=16) and age-matched littermate controls (WT; n=18) were used. All mice received treatment with either N-acetylcysteine (NAC; 40 mg/kg/day), a precursor for glutathione, or saline for a period of 8 weeks via subcutaneously implanted minipumps. At study end, cardiac and renal structure, function, and fibrosis, renal gene expression, and renal glutathione content were assessed in all mice. In a separate cohort, these data were collected in 18-week-old mice (WT; n=16; DCM; n=17) for the evaluation of baseline parameters.

**Results:** At baseline, mean LV wall thickness and ejection fraction were 23% and 36% lower, respectively, in DCM mice than WT ( $P < 0.001$ ). This was accompanied by 88%, 73% and 40% greater levels of tubulointerstitial fibrosis, glomerular fibrosis, and renal oxidized glutathione content, respectively, in DCM mice compared to WT ( $P \leq 0.05$ ). After 8 weeks of treatment, renal oxidized glutathione content was 52% less in NAC-treated DCM mice than untreated DCM mice ( $P < 0.01$ ). This was associated with 99% and 70% less tubulointerstitial and glomerular fibrosis, respectively, in NAC-treated DCM mice compared to untreated DCM mice ( $P < 0.001$ ). Of note, tubulointerstitial and glomerular fibrosis were lower in NAC-treated DCM mice compared to levels at baseline ( $P < 0.05$ ). Lastly, glomerular filtration rate (GFR) was 38% greater in NAC-treated DCM mice compared to untreated DCM mice ( $P < 0.01$ ). Importantly GFR in NAC-treated DCM mice was comparable to that of age-matched WT mice ( $P = 0.99$ ). Treatment with NAC, however, had no effect on cardiac structure or function in DCM mice ( $P \geq 0.20$ ).

**Conclusion:** NAC therapy reversed renal fibrosis and improved renal function in mice with DCM, potentially by restoring the reduced renal glutathione content.

## CLINICAL DECISIONS ON HYPERTENSION MANAGEMENT WILL DIFFER ON THE BASIS OF BRACHIAL VERSUS CENTRAL BLOOD PRESSURE

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**Background:** Brachial blood pressure (BP) is the reference standard for hypertension management, but central systolic BP may significantly differ from brachial systolic BP. The extent to which clinical diagnosis and management may diverge on the basis of brachial versus central BP is uncertain at the population level.

**Aims:** To determine differences in the diagnosis of hypertension in the community, based on brachial and central BP values.

**Methods:** Brachial and central BP (average of 3 recordings) were measured using cuff oscillometry (SphygmoCor<sup>®</sup>-XCEL) among 890 adults participating in a community-screening program. Brachial BP status was determined according to guideline thresholds (i.e.,  $\geq 140/90$  mmHg) and central BP status according to published limits (i.e.,  $\geq 130/90$  mmHg). Theoretical differences in clinical diagnosis were derived from threshold differences between brachial BP and central BP.

**Results:** Participants were aged  $47 \pm 18$  (mean  $\pm$  SD) years (53% women). Prevalence of hypertension according to brachial BP was 35.5% (n=316), but only 75% (n=237) of these people also had central hypertension, leaving another 25% (n=79) with normal central BP. This mostly affected individuals with grade 1 hypertension (systolic BP 140–159 or diastolic BP 90–99; n=66 from 79; 83.5%) based on brachial BP. On the other hand, most people with normal brachial BP also had normal central BP (n=561 from 574; 97.7%), with only 2.3% (n=13) having central hypertension. The prevalence of hypertension based on central BP was lower than the prevalence based on brachial BP (28.1% vs 35.5%). These patterns were observed irrespective of antihypertensive medication use.

**Conclusion:** A significant proportion of people with hypertension according to brachial BP have normal central BP levels. Thus, hypertension management decisions based on central BP are likely to differ compared with decisions based on brachial BP.

## TARGETING THE ANGIOTENSIN II TYPE 2 RECEPTOR TO MAINTAIN CARDIOVASCULAR PROTECTION IN AGED FEMALES

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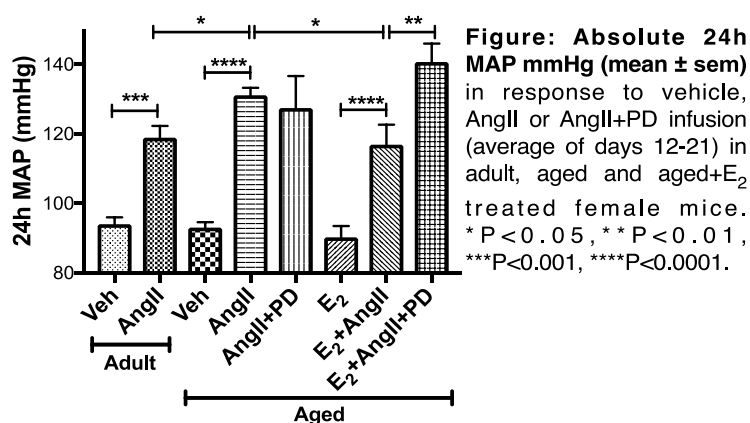
**Background:** Loss of estrogen (E<sub>2</sub>) following menopause contributes to the sharp rise in cardiovascular risk with age. E<sub>2</sub> is postulated to play a protective role against hypertension and end-organ damage by counterbalancing the pressor actions of the renin-angiotensin system (RAS) and enhancing the depressor RAS pathways.

**Aims:** To determine whether E<sub>2</sub> replacement in aged females can lower arterial pressure and improve endothelial function via an angiotensin II type 2 receptor (AT<sub>2</sub>R)-mediated mechanism.

**Methods:** MAP was measured via telemetry in ovary-intact adult (3–4 month old), aged (15–18 month old) and aged+E<sub>2</sub> (3 µg/day, s.c.) FVB/N female mice, which were co-treated with vehicle, Ang II (600 ng/kg/min, s.c.) or Ang II+PD (PD123319, AT<sub>2</sub>R antagonist, 3 mg/kg/day, s.c.). On day 21 of treatment, endothelium-dependent relaxation in response to acetylcholine was assessed in aortic vessels. Cardiac and renal, tissue fibrosis and mRNA levels of E<sub>2</sub> receptors and RAS components were also analyzed.

**Results:** Basal MAP was lower in E<sub>2</sub>-treated aged mice (90±1 mmHg, n=20) relative to adult (94±1 mmHg, n=10) and aged controls (94±1 mmHg; n=21), both *P* < 0.05. The Ang II pressor response was enhanced in aged compared to adult females (Figure). E<sub>2</sub> treatment reduced the Ang II pressor response in aged females (Figure). Moreover, the attenuated pressor response observed in the aged E<sub>2</sub>+Ang II group was abolished by co-infusion with PD (Figure). Endothelial function was impaired with age (~40% reduced maximal vasodilation; *P* = 0.01) and not exacerbated with Ang II infusion. E<sub>2</sub> replacement did not significantly improve the response in any group.

**Conclusion:** E<sub>2</sub> replacement may reinstate cardio-protection in aged females via an AT<sub>2</sub>R-mediated mechanism, and may therefore serve as a novel therapeutic to target post-menopausal hypertension.



## MEASUREMENT OF DIASTOLIC BLOOD PRESSURE IN CHILDREN AND ADOLESCENTS AT REST AND AFTER EXERCISE: 4TH VS 5TH KOROTKOFF PHASE

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**Background:** Blood pressure is measured as part of exercise testing to assess cardiovascular responses to stress. In children and adolescents, there is controversy about whether the 4<sup>th</sup> or 5<sup>th</sup> Korotkoff phase (K4, K5) should be used to define diastolic blood pressure. This controversy mainly arises from known difficulties in detecting K4 or K5 in some children, namely, where no obvious distinction of sound muffling exists or audible sounds continue to zero, respectively. Anecdotally, these problems may be exacerbated following exercise.

**Aims:** To investigate the effect of exercise on the frequency of K4/K5 absence, and to identify associations of exercise performance and age with this phenomenon.

**Methods:** We recruited 90 patients aged 5–18 years who were scheduled for an exercise test at the Royal Children's Hospital Cardiology clinic to the study. Brachial blood pressure was measured at rest and immediately following treadmill exercise (with the Bruce protocol) using the auscultatory method by sphygmomanometry. The cohort was divided into subgroups based on exercise performance ('poor' <25<sup>th</sup> percentile for age/sex, n=40; and 'good' >25<sup>th</sup> percentile, n=50) and age ('children' < 13 years of age, n=45; 'adolescents' ≥ 13 y, n=45).

**Results:** Where measurable, K4 vs. K5 diastolic pressures were 65±9 mmHg (range 45–80 mmHg) vs. 56±14 mmHg (10–80 mmHg; 3 cases < 30 mmHg) at rest and 62±13 mmHg (40–100 mmHg) vs. 53±18 (5–80 mmHg, 3 cases < 30 mmHg) after exercise. K4 was detected in all cases at rest and in all but 3 cases after exercise. K5 could not be detected in 4.4% of cases at rest and 36.7% after exercise ( $P < 0.001$ ). An inability to detect K5 was more frequent in the subgroup with good exercise performance (56.0% good vs. 12.5% poor;  $P < 0.001$ ), and this coincided with a higher mean change in heart rate (116±16 vs. 99±22 bpm;  $P < 0.001$ ). K5 was also more frequently absent in adolescents than children (46.7% vs. 26.7%;  $P = 0.05$ ).

**Conclusion:** K5 was frequently undetectable after exercise, whereas K4 was detectable in the majority of children and adolescents before and after exercise. The frequency of K5 absence was also elevated with better exercise performance and in adolescents compared with children. Results of this study support the use of K4 to define diastolic blood pressure, as it is more reliably present than K5, particularly after exercise. Further studies are, however, needed to establish the accuracy of K4-based diastolic blood pressure in children under these conditions.



## DIETARY NITRATE: NOVEL, INNOVATIVE ROLES IN COMMON, DIVERSE, CARDIOMETABOLIC DISORDERS

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**Background:** Nitric oxide (NO) is a systemic- and pulmonary-vasodilator. *NO synthesis in vivo can be facilitated in vivo by reduction of dietary nitrate (NO<sub>3</sub><sup>-</sup>) to NO independent of NO synthase in a process that is upregulated under certain clinical conditions, possibly providing therapeutic effect.* Multiple cardiometabolic pathologies are associated with perturbations in NO, including hypertension (HTN) and obstructive sleep apnoea syndrome (OSAS). To extend findings from our preliminary studies, we hypothesized that dietary NO<sub>3</sub><sup>-</sup> may have utility in cardiometabolic disorders associated with decreased NO bioavailability and elevated blood pressure (BP).

**Aims:** To assess the effect of daily dietary nitrate on ambulatory blood pressure in difficult to treat cardiometabolic disorders.

**Methods:** We conducted 2 separate double-blind, randomized, placebo-controlled, crossover trials of daily NO<sub>3</sub><sup>-</sup> supplementation as concentrated beetroot juice compared to matching nitrate-depleted beetroot juice placebo (PL) for 7d among a group of well-characterized, uncontrolled hypertensives and subjects with newly diagnosed OSAS on ambulatory BP and biochemical parameters.

**Results:** We recruited 20 uncontrolled hypertensives (mean age=63y, mean BMI=31kg/m<sup>2</sup>, mean no. of antihypertensives=2) as well as 12 adults with severe OSAS (mean apnoea-hypnoea index=74, mean age=52y, mean BMI=31kg/m<sup>2</sup>). Assessments were conducted on three occasions, baseline (day 1), midpoint, (day 8) and endpoint (day 15) – before and after each intervention period and included plasma nitrate as well as 24h ambulatory blood pressure monitoring (table 1).

**Table 1:**

	Baseline	Post- NO <sub>3</sub> <sup>-</sup>	Post-PL	P-value
#Nitrite (nM)	126	+578	+44	0.0017
#24h SBP (mmHg)	137	-8	-4	0.012
#24h DBP (mmHg)	80	-4	-1	0.018
*Nitrite (nM)	65.5	+232	+13	0.0012
*24h SBP (mmHg)	134	-6	-1	0.018
*24h DBP (mmHg)	81	-2	-1	0.09
*Night SBP (mmHg)	127	-8	+1	0.045
*Night DBP (mmHg)	78	-6	-4	0.035

P-values are derived from paired t-tests of the difference between  $\Delta$ NO<sub>3</sub> and  $\Delta$ PL.

# = HTN; \* = OSAS

**Conclusions:** Daily dietary nitrate was well-tolerated, safe and led to increased plasma NO metabolites and decreased BP profiles in uncontrolled hypertensives and OSAS. Dietary nitrate has potential as a novel therapeutic, adjunct strategy in difficult to treat BP. Considering the low cost and safety profile of dietary nitrate containing foods and supplements, this concept appears promising as an adjunct therapeutic strategy for cardiovascular diseases.

## ANALYSIS OF POLYMORPHISMS IN 47 GENES DIFFERENTIALY EXPRESSED DURING CALORIC RESTRICTION FOR ASSOCIATION WITH HUMAN LONGEVITY

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**Background:** Longevity is a polygenic trait in which genetic predisposition is particularly important. We hypothesized that amongst genes differentially expressed in response to caloric restriction in mice, several may be candidate longevity genes in humans.

**Aims:** To test single nucleotide polymorphisms (SNPs) in genes differentially expressed during caloric restriction in mice for association with human longevity.

**Methods:** Subjects were American men of Japanese ancestry recruited in the mid-1960s for the Honolulu Heart Program and followed until the present or death as the Honolulu-Asia Aging Study. The longevity group in the present study comprised 440 men who survived to age  $\geq 95$  years and 374 men who had an average lifespan. Using leukocyte DNA from blood collected in 1991–1993, we tested 459 SNPs in the human homologues of 47 genes found to be differentially expressed in calorically-restricted mice (Estep *et al.* *PLoS One* 2009;4:e5242) and 11 other genes of interest (*RPTOR*, *RICTOR*, *SIRT1*, *SIRT2*, *SIRT3*, *SIRT4*, *SIRT5*, *SIRT6*, *LMNA*, *APOE/TOMM40*) for association with longevity. All SNPs chosen had a minor allele frequency  $\geq 5\%$ . SNPs were genotyped at the University of Hawaii Cancer Center on the Illumina GoldenGate platform, which performs high-throughput SNP genotyping on universal bead arrays.

**Results:** Based on a dominant model of inheritance, an association with longevity at the  $P < 0.05$  level was seen for SNPs in 13 of the genes. Testing by all possible models increased the number of genes to 16. After correction for multiple testing, SNPs in 4 genes retained significance, namely, *MAP3K5* ( $P=0.00004$ ), *SIRT7* ( $P=0.00004$ ), *SIRT5* ( $P=0.0007$ ), and *PIK3R1* ( $P=0.01$ ). In a dominant model, association with longevity was seen for multiple adjacent SNPs within two of these (*MAP3K5* and *PIK3R1*), as well as in *FLT1*, consistent with linkage disequilibrium with a causative variant in the vicinity of each respective SNP set. Haplotypes of *MAP3K5* and *FLT1* were associated with longevity.

**Conclusion:** The present study implicates variation in *MAP3K5*, *FLT1*, *PIK3R1*, *SIRT7* and *SIRT5* in human longevity. These may merit further study in other populations and age-related conditions.

## SPECIFICITY OF DNA METHYLATION PATTERNS IN THE HYPERTENSIVE KIDNEY

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**Background:** Evidence suggests that DNA methylation (5mC) is important in the development of essential hypertension (EH). The 5mC percentage, a measurement for global methylation studies, in peripheral blood leukocytes (PBL) has been previously associated with hypertension. Methylation patterns are tissue-specific, contributing to differences in transcriptional regulation and cellular differentiation. So far, there have been no studies of 5mC in the kidney – an important effector organ in EH. Furthermore, there has been no investigation of the relationship between 5mC patterns in the hypertensive kidney and PBLs.

**Aims:** (i) To determine if global 5mC in the kidney is correlated to hypertension diagnosis and blood pressure (BP) regulation. (ii) To determine whether PBLs provide a surrogate for cross-tissue patterns of 5mC in the kidney.

**Methods:** We used 96 human kidney and 76 human PBL samples from the TRANSLATE study to investigate global 5mC percentage. TRANSLATE consists of carefully characterized collections of “apparently healthy” specimens of human kidneys. Global methylation was determined using the 5mC ELISA kit (Zymo Research) that measures the total amount of 5mC present in a sample.

**Results:** We found no association of global 5mC percentage in kidney ( $P=0.18$ ) and PBL ( $P=0.54$ ) with hypertension diagnosis, nor between PBL 5mC percentage and BP. However, a negative correlation was found between kidney 5mC percentage and systolic BP ( $r=-0.246$ ;  $P<0.05$ ), and diastolic BP ( $r=-0.319$ ;  $P<0.01$ ). This association was still evident after adjustment for antihypertensive medication for systolic BP ( $r=-0.210$ ;  $P<0.05$ ) and diastolic BP ( $r=-0.273$ ;  $P<0.01$ ). Furthermore, we found a strong positive correlation between normotensive kidneys and leukocyte 5mC percentages ( $r=0.864$ ;  $P<0.01$ ). Similarly, a strong positive correlation was evident for hypertensive kidneys and leukocyte 5mC percentages ( $r=0.916$ ;  $P<0.01$ ).

**Conclusion:** Our findings show that kidney 5mC, but not PBL 5mC, is correlated to BP regulation. No relationship was evident for global 5mC and hypertension diagnosis, regardless of the tissue type studied. Furthermore, PBL 5mC global methylation percentage was highly correlated to kidney 5mC percentage. These results highlight the importance of further studies on the involvement of kidney DNA methylation in hypertension, as well as further investigation of the relationship between methylation patterns in the kidney and blood.

## RESTORING IMPAIRED L-ARGININE TRANSPORT CAN ATTENUATE RENAL FIBROSIS AND INFLAMMATION IN A MOUSE MODEL OF DILATED CARDIOMYOPATHY

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**Background:** Heart failure (HF) affects more than 28 million people worldwide. Approximately 45% of these patients develop renal dysfunction, and this is associated with higher morbidity and mortality. Impaired L-arginine transport has been implicated in cardiac and renal failure. In diseased states, the transport of L-arginine into endothelial cells is impaired. This augments nitric oxide (NO) production, which can induce development of fibrosis.

**Aims:** To determine if augmenting L-arginine transport by increasing expression of its transporter (cationic amino acid transporter 1; CAT1) can attenuate renal fibrosis and inflammation in the setting of HF.

**Methods:** Eighteen-week-old WT mice (n=8), transgenic mice with dilated cardiomyopathy (DCM; n=8), and double transgenic mice with DCM and endothelial-specific overexpression of CAT1 (HFCAT1; n=8) were studied. Plasma nitrate/nitrite levels, cardiac and renal fibrosis, mRNA expression, structure and function were assessed in all mice.

**Results:** Plasma nitrate/nitrite was 78% less in DCM mice than WT ( $P < 0.05$ ). This was restored in HFCAT1 mice to levels observed in WT. Cardiac interstitial and perivascular fibrosis were 89% and 45% greater, respectively, in DCM mice than WT ( $P < 0.001$ ). Tubulointerstitial and glomerular fibrosis were 89% and 76% greater, respectively, in DCM mice than WT ( $P < 0.05$ ). Cardiac and renal fibrosis were significantly attenuated in HFCAT1 mice compared to DCM mice ( $P < 0.05$ ). Consistent with this, renal expression of *Il6* and *Il1 $\beta$*  mRNAs were less in HFCAT1 mice than DCM ( $P < 0.05$ ). Renal expression of *Il10* and *Cat1* mRNAs were 57% and 23% less in DCM mice than WT and these were restored in HFCAT1 mice ( $P < 0.05$ ). Cardiac expression of *Il6* mRNAs was 92% greater in DCM mice than WT and this was normalized in HFCAT1 mice ( $P < 0.01$ ). Mean LV wall thickness and ejection fraction were 23% and 33% less, respectively, in DCM mice compared to WT ( $P < 0.01$ ). Endothelial-specific overexpression of *Cat1* mRNA in DCM mice had no effect on mean LV wall thickness and ejection fraction ( $P > 0.24$ ). Endothelial-specific overexpression of *Cat1* mRNA in DCM mice also had no effect on renal function ( $P > 0.98$ ).

**Conclusion:** Augmenting L-arginine transport by increasing expression of *Cat1* can attenuate renal fibrosis and inflammation in the setting of HF.

## FROM POLYGENIC TO OMNIGENIC: DIRECT GENE-GENE INTERACTIONS INVOLVING FOXO3 AT THE HUB OF A 46-GENE CELL RESILIENCE “GENE FACTORY” ON HUMAN CHROMOSOME 6

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**Background:** Protective alleles of *FOXO3* single nucleotide polymorphisms (SNPs) are associated with lower blood pressure, less hypertension and longevity, principally via protection against coronary artery disease mortality (Willcox *et al. Aging Cell* 2016;15:617-24). We have shown that *FOXO3*, via CCCTC-binding factor zinc finger protein binding sites, engages in long-range interactions with 46 neighbouring genes in a 7.3 Mb gene cluster (topologically associated domain) at chromosome 6q21 and that *FOXO3* is at the central hub of this cell-resilience “gene factory” (Donlon *et al. Aging Cell* 2017;16:1016-25). This appears to be a possible example of a mechanism that accords with the recent omnigenic model invoked to explain the “missing heritability” of complex polygenic traits (Boyle *et al. Cell* 2017;169:1177-86).

**Aims:** To visualize *FOXO3* gene-gene interactions and elucidate the local genomic mechanisms involved.

**Methods:** Using qRT-PCR we measured *FOXO3* mRNA from lymphoblastoid cell lines cultured for 24 h in the presence or absence of a stressful stimulus (200  $\mu$ M H<sub>2</sub>O<sub>2</sub> + serum deprivation). Cell lines were derived from 20 offspring of long-lived Japanese American subjects; 10 were heterozygous for the protective (G) allele of *FOXO3* SNP *rs2802292* and 10 were homozygous for the common (T) allele. Fluorescently-labelled BAC clones were used for fluorescent *in situ* hybridization (FISH). The WashU Epigenome Browser public database and the program Juicebox were used to determine contact points for interactions.

**Results:** Cell stress increased *FOXO3* mRNA levels. *FOXO3* activation was 3-fold stronger in cells carrying the resilience-associated G-allele of SNP *rs2802292*. The gene *HACE1* (3 Mb proximal to *FOXO3*) was also expressed at higher levels following cellular stress. There was, however, no genotypic difference. In FISH experiments, stress-induced activation of *FOXO3* caused it to move towards *HACE1* and *LAMA4*, which are located at either distal flanking end of the 46-gene neighbourhood. *FOXO3* also moved towards genes *ATG5* and *AMD1* that are located closer to *FOXO3*. We mapped 628 long non-coding RNAs (lncRNAs) to the 6q21 region. These included *WISP3* and *TUBE1*. A putative lncRNA binding site was present in the *FOXO3* promoter. Several neighbouring lncRNAs (*OSTM1*, *SNX3*, *LINC00222*, and *CCDC162P*) appeared to be connected with the *FOXO3* promoter and *FOXO3* longevity-associated SNPs via RNA polymerase II binding. We hypothesize that at least some of these lncRNAs may be involved in *FOXO3* interactions and complex formation with neighbouring genes.

**Conclusion:** *FOXO3* mobilizes neighbouring genes by gene-gene interactions. These might involve lncRNAs in the region. We believe that gene-gene interactions amplify *FOXO3*'s genetic effect and fits with what we propose is a novel aspect of the recent omnigenic model invoked to explain “missing heritability” in complex polygenic conditions such as essential hypertension and longevity.

## NUTRITIONAL STRATEGIES TO PREVENT AND TREAT HEART FAILURE: A COMPREHENSIVE REVIEW OF HUMAN STUDIES

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**Background:** Nutrition is regarded as a major modifier of the incidence and severity of cardiovascular diseases (CVD). Compared to other CVD, there is, however, relatively little nutritional research into heart failure (HF). As such, guidelines typically focus on sodium and fluid restriction.

**Aims:** To comprehensively review existing studies regarding dietary patterns, food and dietary components in relation to HF incidence and severity.

**Methods:** A comprehensive search of multiple online databases, using multiple keywords was conducted.

**Results:** The Dietary Approaches to Stop Hypertension (DASH) and Mediterranean diets have consistently been associated with decreased HF prevalence, incidence and severity. Regarding specific dietary components, fruit, vegetables, legumes and whole-grains appear to be beneficial, while red/processed meats, eggs and refined carbohydrates appear harmful. Fish, dairy and poultry remain controversial. The existing but limited observational and interventional evidence from human studies suggest that a plant-based dietary pattern high in antioxidants, micronutrients, nitrate and fibre, but low in saturated/trans fat and sodium, may decrease HF incidence/severity. Potential mechanisms include decreased oxidative stress, homocysteine and inflammation, but higher anti-oxidant defence and nitric oxide bioavailability and gut microbiome modulation.

**Conclusions:** Existing evidence suggests that the potential for nutrition to help prevent and treat HF is large yet vastly under-estimated. There is, however, a notable lack of human intervention trials. Cardiac clinicians should be made aware of the large, mostly consistent, evidence regarding nutrition and HF.

**SEVENTEEN WAYS TO TREAT HYPERTENSION**

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Almost a century after Dr. Harry Goldblatt published the first animal model of hypertension, we are still facing a hypertension epidemic with over one-third of the world's population afflicted with this disease. Although the etiology of most cases of adult hypertension remains poorly defined, it is generally accepted that sustained hypertension requires a combination of impaired renal excretory capacity and increased peripheral vascular resistance. Interestingly, immune cells, including T cells and monocytes/macrophages, have long been observed in vessels and kidneys of hypertensive animals and humans, but their role has not been fully appreciated until recently. Work from our Laboratory and others has revealed that several inflammatory cytokines released by these cells have important effects on adjacent parenchymal cells. This lecture will focus on one particular cytokine, interleukin-17 (IL-17). The speaker first demonstrated that mice lacking this cytokine develop blunted hypertension and have preserved vascular function in response to chronic angiotensin II infusion. Recently, we found that gamma delta T lymphocytes and T helper 17 (Th17) cells are the predominant immune sources of IL-17 in the kidney and blood vessels in hypertension, but that renal epithelial cells can also produce IL-17. The salt-sensing kinase, serum and glucocorticoid regulated kinase 1 (SGK1), plays an important role in both T lymphocytes and the kidney to regulate blood pressure. Using a combination of genetic deletion and/or antibody mediated neutralization approaches targeting SGK1, IL-17, or its receptor, we identified a pathway by which T cell SGK1 controls IL-17 production and promotes renal/vascular inflammation in hypertension, and that IL-17 in turn regulates renal sodium transporters through an SGK1 dependent pathway and alters renal/vascular function leading to elevated blood pressure. Thus, SGK1, IL-17, or its receptor may be potential novel therapeutic targets for the treatment of hypertension and the accompanying end-organ damage

## HYPERTENSION AND DIABETES DIFFERENTIALLY AFFECT RENAL REACTIVE OXYGEN SPECIES AND CATECHOLAMINE CONTENT

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**Background:** Patients with both diabetes and hypertension develop nephropathy at an accelerated rate. Using the hypertensive Schlager mouse model, we examined changes in renal function, sympathetic nerve status and the oxidative status of the kidney in diabetic mice with and without concomitant hypertension.

**Aims:** To examine changes in renal function, sympathetic nerve status and the oxidative status of the kidney in diabetic mice with and without concomitant hypertension.

**Methods:** After 10 weeks of study, hypertensive BPH/2J and normotensive BPN/3J Schlager mice with and without concomitant streptozotocin-induced diabetes (5x 55 mg/kg, i.p.) were placed in metabolic cages for 24 h and kidneys were harvested. In a separate group of animals BP telemetry probes were implanted.

**Results:** Induction of diabetes did not change the hypertensive status of BPH mice (MAP 131±4 vs. 129±4 mmHg for non-diabetic vs. diabetic BPH, n=5 and 6, respectively). Diabetic BPN and BPH mice showed significantly greater albuminuria than non-diabetic controls, with diabetic BPN showing less albuminuria than diabetic BPH (439±73 vs. 1205±196 µg/24 h, n=8, 7). Plasma cystatin C was significantly lower in diabetic animals, with no difference between strains. HPLC measurement of cortical noradrenaline showed significantly greater levels in kidneys from hypertensive mice, but, interestingly, diabetic mice had significantly less renal noradrenaline. Renal cortical peroxide formation was increased in non-diabetic BPH mice and while activity of the anti-oxidant enzyme catalase was increased in non-diabetic BPH mice it was significantly less in diabetic BPH animals (non-diabetic vs. diabetic BPH 104±8 vs. 63±6 nmol/min/ml, n=8/group).

**Conclusion:** Kidneys of non-diabetic hypertensive mice show greater renal oxidative stress than normotensive mice. While diabetic hypertensive animals have greater oxidative stress, they had lower catalase activity, indicating compromised ability to deal with hypertension leads to increases in oxidative stress. This could contribute to greater renal neuropathy, further compromising renal function. This mechanism may underlie the poor outcome for patients with hypertensive diabetic nephropathy.



## NITRIC OXIDE ASSOCIATED PROTEINS OF VASCULAR ENDOTHELIAL CELLS ARE MODIFIED BY RATE OF PULSATILE STRETCH

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**Background:** Endothelial nitric oxide synthase (eNOS) is generated through pulsatile stretch of endothelial cells through phosphorylation of eNOS, releasing nitric oxide (NO) and increasing cyclic guanosine monophosphate (cGMP). Although cyclic stretch-mediated phosphorylation of eNOS at serine 1177 is known to be regulated by phosphorylated Akt at S473 and activated intercellular cell adhesion molecule-1 (ICAM-1), the effect of frequency (simulating changes in heart rate) with stretch intensity has not been established in endothelial cells.

**Aims:** To investigate the effect of frequency of pulsatile stretch on eNOS, ICAM-1, protein kinase B (Akt) expression and phosphorylation and downstream products of Akt signalling such as NO and cGMP in vascular endothelial cells.

**Methods:** Human umbilical vein endothelial cells (HUVECs) were stretched cyclically at 0.5 or 1 Hz at a magnitude of 5–20% for 18 hours. Specific proteins and mRNAs were quantified using Western blotting and qPCR, respectively. NO and cGMP were quantified using commercial assay kits.

**Results:** eNOS protein was higher at 1 Hz than at 0.5 Hz ( $66\pm 11\%$  and  $211\pm 37\%$ , respectively;  $P=0.0006$ ) as was ICAM-1 ( $69\pm 5\%$  and  $210\pm 47\%$ , respectively;  $P=0.0079$ ). Phosphorylated eNOS (S1177), phosphorylated Akt, eNOS mRNA and NO changed when endothelial cells were stretched compared to endothelial cells not stretched, but there was no effect of change in cycling frequency. There was no change in cGMP.

**Conclusion:** Results demonstrate eNOS activation might not be entirely regulated by Akt/PKB pathway, and differential expression of proteins related to NO production in HUVECs is mediated by pulsatile stretch at 0.5 and 1 Hz.

## PULSATILE STRETCH LEADS TO ELEVATED AMYLOID BURDEN AND IMPAIRED NITRIC OXIDE SIGNALLING IN HUMAN CEREBRAL MICROVASCULAR ENDOTHELIAL CELLS

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**Background:** Amyloid  $\beta$  ( $A\beta$ ) aggregates are characteristic of Alzheimer's disease (AD).  $A\beta$  originates from sequential cleavage of amyloid precursor protein (APP) by proteolytic enzymes such as  $\beta$ -secretase 1 (BACE-1). An inverse relationship between amyloid burden and nitric oxide (NO) bioavailability has been reported in endothelial nitric oxide synthase (eNOS) knockout mice. Elevated pulsatility of arterial pressure and NO signalling are independently associated with AD.

**Aims:** To investigate the effect of pulsatile stretch of human cerebral microvascular endothelial cells (hCMEC) on the expression and processing of APP and expression and phosphorylation of eNOS

**Methods:** hCMECs were subjected to 5%, 10% and 15% cyclic stretch for 18 hours at 1 Hz and were compared to the control (0% stretch) to evaluate the effect of pulsatility on APP, BACE-1,  $A\beta$ , eNOS and phosphorylated eNOS (phospho-eNOS) at serine 1177. APP, eNOS and phospho-eNOS were quantified by western blotting ( $n=5-11$ ).  $A\beta_{42}$  secretion was measured using ELISA. Results were analyzed using one-way ANOVA and are represented as mean  $\pm$  SEM.

**Results:** APP expression was significantly higher at 10% ( $240\pm 52\%$ ;  $P < 0.05$ ) and 15% ( $265\pm 34\%$ ;  $P < 0.05$ ) of pulsatile stretch magnitudes compared to the static control (100%). Consistently, expression of APP processing enzyme, BACE-1, was also increased at 10% ( $239\pm 32\%$ ;  $P < 0.05$ ) and 15% ( $242\pm 52\%$ ;  $P < 0.05$ ) stretch magnitudes while secreted  $A\beta_{42}$  showed a significant positive linear relationship with increasing magnitude of pulsatile stretch ( $r^2 = 0.21$ ;  $P < 0.01$ ). Protein expression of eNOS was significantly higher at 15% stretch magnitude ( $248\pm 66\%$ ) relative to the 0% static control (100%;  $P < 0.05$ ). In contrast to the upregulated eNOS protein expression levels, the phosphorylation of eNOS at serine-1177, a common activation site of eNOS, was downregulated at 5% ( $70 \pm 11\%$ ;  $P < 0.05$ ), 10% ( $46 \pm 8\%$ ;  $P < 0.0001$ ) and 15% ( $23 \pm 3\%$ ;  $P < 0.0001$ ) stretch magnitudes relative to the 0% static control (100%). Phospho-eNOS level at the 5% stretch magnitude ( $70 \pm 11\%$ ;  $P < 0.001$ ) was significantly higher than that at the 15% stretch ( $23 \pm 3\%$ ).

**Conclusion:** Pulsatile stretch of cerebral ECs lead to an increase in APP, BACE-1 and  $A\beta_{42}$  levels. Downregulation of phospho-eNOS at the common activation site of eNOS, S1177 consequent of stretch indicated decreased bioavailable NO reservoirs. These findings mechanistically support microvascular AD pathology related to increased cyclic stretch associated with elevated pulse pressure.

## APPLICATION OF A NOVEL ANGIOTENSIN II ASSAY, INCLUDING A COMPARISON OF RENIN, ANGIOTENSIN II AND ALDOSTERONE IN RESPONSE TO UPRIGHT POSTURE STIMULATION IN PATIENTS WITH AND WITHOUT PRIMARY ALDOSTERONISM

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**Background:** Primary aldosteronism (PA), considered the most common cause of endocrine hypertension, is characterized by excessive aldosterone production which is relatively autonomous of its main regulator –renin/angiotensin II, levels of which are usually suppressed. Although aldosterone/renin ratio (ARR) is currently recommended as the most reliable approach in PA screening, many medications (including most antihypertensives) and various physiological factors (posture, menstrual cycle, and dietary sodium, etc.) have been reported to affect renin (more markedly) and aldosterone levels, resulting in false negative or false positive ARR results. Compared to the upstream renin, which has no direct effect on aldosterone secretion, the downstream angiotensin II is the main direct regulator of aldosterone biosynthesis and may have potential to substitute susceptible renin in future case detection of PA.

**Aims:** To validate a novel angiotensin II assay and to compare the changes of peripheral levels of renin, angiotensin II, and aldosterone in response to upright posture stimulation in patients with and without PA.

**Methods:** Blood samples were collected at 7 AM following overnight recumbency and again at 10 AM, 3 h after an assumption of upright posture (sitting, standing or walking), from 48 patients with PA and 20 patients without PA. Aldosterone was defined as being responsive to upright posture (also known as angiotensin II-responsive) if the level at 10 AM was at least 50% higher than at 7 AM. Serum aldosterone concentration (Aldo) was measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Direct renin concentration (DRC) was determined by chemiluminescent immunoassay. Serum equilibrium angiotensin II (eqAngII) was measured using a novel renin-angiotensin system (RAS) equilibrium analysis based on LC-MS/MS.

**Results:** PA patients demonstrated significantly ( $P < 0.01$ ) higher levels of Aldo, the ARR, and the aldosterone/angiotensin II ratio (AA2R) than non-PA patients, and significantly ( $P < 0.01$ ) lower levels of DRC, eqAngII, and  $K^+$  in both recumbent and upright positions, as expected. While DRC was below the lower limit of quantification (LLOQ) of 2 mU/L in 62.5% PA patients and 5.0% non-PA patients, only 4.2% PA patients and 5.0% non-PA patients had eqAngII below the LLOQ of 2 pmol/L. Upright posture stimulation significantly ( $P < 0.01$ ) increased Aldo, DRC and eqAngII in both groups, and 29 (60.4%) PA patients and 10 (50.0%) non-PA patients showed posture-responsiveness in terms of Aldo with average increases of 190% and 170%, respectively. DRC and eqAngII in the upright position showed significantly ( $P < 0.01$ ) positive correlations in both groups, with Spearman correlation coefficients at 0.884 and 0.746, respectively.

**Conclusion:** This study reports the measurement of equilibrium angiotensin II concentration using LC-MS/MS among non-PA and PA patients. EqAngII could be readily quantified by LC-MS/MS and had a broader reference range than DRC. The dynamic changes of DRC and eqAngII showed good consistency in the upright position, indicating that this novel angiotensin II assay may have the potential for future application in the diagnostic workup of PA.

## SALINE SUPPRESSION TESTING MAY PREDICT THE SUBTYPE OF PRIMARY ALDOSTERONISM

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**Background:** The saline suppression test (SST) confirms the diagnosis of primary aldosteronism (PA) in patients with an elevated aldosterone:renin ratio. This is usually followed by adrenal vein sampling (AVS) to define PA subtype as either unilateral (predominantly an aldosterone-producing adenoma) or bilateral (adrenal hyperplasia). In SST, serum electrolytes, aldosterone, cortisol and renin are measured at baseline and 4 hours after saline infusion. It is unclear if a combination of these parameters can reliably predict bilateral PA, which would be expected to reduce the need for AVS and expedite the patients' medical treatment.

**Aims:** To identify SST parameters that distinguish bilateral from unilateral PA.

**Methods:** A retrospective analysis was performed on 96 patients who underwent the recumbent SST at Monash Health (February 2011 to July 2017). A positive SST was defined as plasma aldosterone concentration (PAC) >140 pmol/L at 4 hours post-infusion of 2 L normal saline in the recumbent position. Patients with positive SST results were categorized into three PA subtypes based on their AVS findings: unilateral, bilateral and undetermined. Results were expressed as median (lower and upper quartiles).

**Results:** A positive SST was seen in 91 out of 96 patients (95%). Of these, 64 patients underwent AVS, which revealed unilateral disease in 25, bilateral disease in 25 and was undetermined in 14 patients. The unilateral group had significantly higher PAC compared to the bilateral group, both at 0 hours (538 pmol/L (441–748) vs. 323 pmol/L (250–429);  $P=0.004$ ) and at 4 hours (462 pmol/L (280–764) vs. 230 pmol/L (195–298);  $P=0.05$ ). Compared to the bilateral group, PAC in the unilateral group demonstrated a lower absolute reduction at 4 hours (–69 pmol/L (–178–30) vs. –87 pmol/L (–142–44)) and a smaller percentage decrease at 4 hours (–17% vs. –27%), although these were not statistically significant. Strikingly, the aldosterone to cortisol ratio (ACR) was significantly higher in the unilateral compared to the bilateral PA group at 4 hours. A combination of PAC < 240 pmol/L and ACR < 1.68 post-SST provided an estimated specificity of 95.8% and a positive predictive value of 92.9% for bilateral PA (95% CI 78.9–99.9 and 66.1–99.8%, respectively).

**Conclusion:** Patients with the bilateral subtype of PA had a lower PAC and ACR following saline infusion. These parameters may help to predict bilateral PA with high specificity. A larger independent sample is, however, required to validate these findings.

## RELATIONSHIP OF CEREBRAL BLOOD FLOW AND CENTRAL AORTIC PRESSURE WITH AGE AND HYPERTENSION

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**Background:** Pulsatile features of the central aortic pressure (cAP) waveform are related to corresponding pulsatile features of flow velocity (FV) in cerebral arteries. However, changes in mean pressure that occur with age and hypertension can affect this relationship.

**Aims:** The aim of this study was to assess the association of changes in pulsatility of cAP and cerebral arterial FV in normotensive subjects and in young and old subjects with hypertension.

**Methods:** The study cohort consisted of three groups of adult subjects: normotensive (NT; n=22, age  $28 \pm 4.8$  yrs); young hypertensive (YHT; n=11, age  $41 \pm 5.4$  yrs) and old hypertensive (OHT; n=12, age  $63 \pm 6.2$  yrs). Hypertension was defined as brachial systolic pressure  $>140$ mmHg or diastolic pressure  $>90$  mmHg. Non-invasive measurements of cAP were obtained from transformation of calibrated radial tonometry waveforms using the SphygmoCor system (AtCor Medical, Sydney). Cerebral FV was recorded by a transcranial doppler device in the middle cerebral artery. Pulsatility was assessed in terms of the ratio of pulse to mean components of cAP (pPI) and cerebral FV (fPI).

**Results:** Mean arterial pressure for NT was  $87.2 \pm 6.6$  mmHg and increased significantly ( $p < 0.05$ ) to  $108.5 \pm 6.1$  mmHg for YHT and  $111.7 \pm 11.8$  mmHg for OHT. However, there were no significant corresponding changes in mean FV (YNT:  $61.1 \pm 11.9$  cm/s; YHT:  $67.2 \pm 17.3$  cm/s; OHT:  $59.6 \pm 20.5$  cm/s). Compared to NT, corresponding mean changes (%) of pPI and fPI were YHT: pPI=20%, fPI=10%; OHT: pPI=88%; fPI=31%.

**Conclusion:** Mean cerebral FV is maintained with changes in mean systemic arterial pressure in both young and old hypertensive subjects, suggesting the presence of autoregulation of the cerebral circulation. In addition, pulsatility of cerebral FV increases with age in hypertensive subjects, but is markedly less than the corresponding increase in pulsatility of cAP.