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PHOTOSTIMULATION OF CHANNELRHODOPSIN2-TRANSFECTED C1 NEURONS ACTIVATES PERIPHERAL SYMPATHETIC VASOMOTOR AFFERENTS AND NEURONS IN THE LOCUS COERULEUS AND A5 REGION IN SPRAGUE DAWLEY RATS

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The C1 neurons of the rostral ventrolateral medulla (RVLM) innervate sympathetic preganglionic neurons in the spinal cord that convey sympathetic vasomotor tone, and the catecholaminergic neurons of the locus coeruleus (LC) and A5 region. In this study we investigated the effects of C1 neuron stimulation on sympathetic vasomotor discharge and the activity of LC and A5 neurons. We used a lentivirus that expresses a fusion protein between the light-sensitive Channelrhodopsin 2 (ChR2) and the fluorescent reporter mCherry under the control of the PRSx8 artificial promoter (a Phox2-responsive element) to drive ChR2-mCherry expression selectively into Phox2b-positive neurons (89 ± 1 % N = 12). Virus microinjections into the C1 region produce ChR2-mCherry expression in C1 neurons and non-C1 Phox2b-positive neurons in the retrotrapezoid nucleus (1:1 ratio of C1: non-C1 neurons, 70% of all C1 neurons). Pulsed photostimulation of the RVLM with 473 nm laser light (LL) in vivo (10 ms pulses, 20 Hz, 30 μW/mm2; intracellular pressure (AP) by 13.8 ± 2.2 mmHg and splanchic sympathetic nerve discharge (sSNND) by 60.3 % (N = 9). Following chemical blockade of the sympathetic baroreflex, photostimulation produced a significantly greater increase in arterial pressure (P < 0.01; N = 6) and SND (P < 0.05; N = 6). Single LL pulses produced a massive evoked burst of SND (peak; 1443 ± 223 % relative to baseline, onset latency: 28 ± 1 ms; N = 9) followed by a long-lasting reduction of SND (18.9 ± 5.5 % relative to baseline). Twin-pulse stimulation revealed a significant reduction in the amplitude of the second pulse if delayed less than 2 s after the initial pulse. Low frequency photostimulation of the C1 region produced a temporally precise activation of both LC (16/22; N = 4) and A5 (3/8; N = 2) neurons. Intracerebroventricular administration of kynurenic acid blocked C1 evoked excitation of LC neurons (12/12). These results confirm that the C1 neurons are sympathetic preganglionic neurons and control AP, but are strongly gated by the sympathetic baroreflex and the properties of sympathetic preganglionic neurons. Furthermore, preliminary data suggests that C1 neurons excite neurons in the A5 and LC, probably through the release of excitatory neurotransmitters.

Estimation of the Pressure Wave in Human Upper Limb

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In volunteers for the Asklepios study, Segers et al (Hypertension 2009:54: 414) described brachial-radial amplification as responsible for most of the amplification of the arterial pressure wave in the upper limb. However, the high amplification in the forearm was different to that recorded from invasive studies in the upper limb by Earl Wood in the 1950s and many investigators since. Since findings from Asklepios study has not been seen in other studies, we repeated the study by calibrating the central aortic pressure (generated from the radial waveform), from both the radial artery waveform and from the brachial waveform, recorded by applanation tonometry. We set systolic peak and diastolic nadir of each to cuff brachial systolic (SBP) and diastolic (DBP) pressure. We then measured the carotid waveform by applanation tonometry, and calibrated this by assuming that mean (MP) and DP were the same as in the arm.

Studies were undertaken in 70/27 women) volunteers and patients (mean age 70 years SD 14.9) at a clinic site.

Results were broadly in line with the Asklepios study, with amplification of the pulse greatest in the forearm (8.8 (SD 7.5) mmHg, and negligible in the carotid-brachial segment (0.3 mmHg SD 6.4). MP calculated from arithmetic integration of the pressure wave over 8 to 10 cycles enabled determination of Form Factor (FF) – the proportion of pulse pressure needed to be added to diastolic pressure for generation of mean pressure. FF (%) was virtually identical in the brachial artery (39.3 SD 5.1) and carotid artery (39.7 SD 3.9), and quite different to that for the radial artery (34.5 SD 3.9). The brachial waveforms recorded by tonometry were different to the radial, with a blunt peak, and different to waveforms measured invasively in previous studies. Applanation of the brachial artery against bone could not be ensured through the bicipital aponeurosis. High values of FF for the brachial and carotid artery were similar to those of Asklepios group, and correspond to the low amplification of the pulse between carotid and brachial sites.

The current method of calibrating the radial waveform to brachial cuff pressure is more appropriate than using brachial tonometry. Invasive studies of simultaneously recorded brachial and radial waveform will be necessary to confirm or refute this view.

Acute Administration of Chlorogenic Acid Reduces Blood Pressure in the Rat

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Hypertension is a major risk for cerebro- and cardio-vascular diseases. Although elevated blood pressure (BP) is usually controllable by therapeutic means, diet based strategies to lower BP are becoming increasingly popular, as they may act as a safer and more cost-effective alternative when compared to conventional drug therapy. Recent efforts have identified potential therapeutic roles in human health for specific dietary components (bioactives). For example, several bioactive peptides which inhibit ACE and/or antagonise the AT1 receptor have been isolated, and products consequently formulated. In addition, bioactives. For example, several bioactive peptides which inhibit ACE and/or antagonise the AT1 receptor have been isolated, and products consequently formulated. In addition, such a process can be used (when relevant aortic diameter is known) to estimate of stroke volume and cardiac output, and was studied in this report.

Flow velocity waveforms in the left ventricular outflow tract were measured by Doppler from the aortic valve in 33 patients admitted for cardiac catheterisation with suspected coronary artery disease. Within a 10 to 15 minutes period, radial artery tonometry was applied and used to generate ascending aortic pressure wave waves using the SphygmoCor® TF.

Ascending aortic impedance values in humans, and their change with age, were determined from the literature and incorporated into a model which used this as age-dependent TF so as to convert aortic pressure harmonic moduli to corresponding aortic flow moduli; the flow wave was synthesised from the first 5 harmonics with flow >1 cm/s. The formula was: Flow (cm/s) = Pressure (dyne cm−2) / Impedance (dyne s.cm−2).

Flow waveforms at different ages were realistic, with peak flow corresponding to the first systolic pressure peak, and with flow essentially zero throughout diastole – as in the Doppler flow waveforms. Numerical values were also realistic with peak flow averaging 64 cm/s (calculated) c.f. 107 cm/s measured by Doppler; stroke volume averaging 56 ml (calculated) c.f. 81 ml (measured by Doppler), and cardiac output averaging 3.7 L/min (calculated) c.f. 5.1 L/min (measured by Doppler).

The double TF method (pressure – pressure then pressure – flow), is physiologically appropriate under control conditions, and appears to provide realistic flow from the heart, calculated from pressure waveforms and modelled impedance. Systematically lower calculated values may be true, and due to measurement of Doppler flow velocity from the outer envelope of the wave.
MEASUREMENT OF MEAN ARM PRESSURE FOR CALCULATION OF CENTRAL AORTIC SYSTOLIC AND PULSE PRESSURE

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In 1992, Kelly and Fitchett [JACC 1992:20:652] proposed that the carotid pressure waveform, measured non-invasively by applanation tonometry, could be calibrated from upper arm mean and diastolic pressure, with the assumption that these values were identical in central and peripheral vessels. This has been widely used since but the practice has been questioned when mean pressure (MP) is calculated from diastolic pressure plus a fixed proportion (Factor FF) of systolic pressure (PP) [Hypertension 2009;54:414]. This study was undertaken to establish in a clinical database, the range of FF in tonometric radial artery waveforms, and in aortic waveforms calculated from a generalised aortic-radial transfer function, and its dependence on age, MP, heart rate (HR), oscillation duration (ED), PP amplification (PPA), brachial PP and aortic augmentation index (AIx). The database [JAHSH 2008;6:22] comprised 5221 observations in 1550.

Radial artery FF was 33.6%, varying from 17.6% to 50.9% with SD 4.4%. Aortic FF was higher (P<0.001) at 41.0%, varying from 25.0% to 60.9% with SD 4.2%. FF at both sites were similar in males and females. There was a significant (P<0.001) relationship between radial FF and age, MP, HR, ED, PPA, PP and AIx.

<table>
<thead>
<tr>
<th>Mean</th>
<th>SD</th>
<th>R² for correlation</th>
<th>Regression</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radial FF (%)</td>
<td>33.6</td>
<td>4.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>69.4</td>
<td>13.2</td>
<td>0.004</td>
<td>-0.02</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>66.6</td>
<td>12.7</td>
<td>0.009</td>
<td>0.034</td>
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<td>ED ( msec)</td>
<td>318.1</td>
<td>32.4</td>
<td>0.077</td>
<td>0.038</td>
</tr>
<tr>
<td>MP (mmHg)</td>
<td>95.3</td>
<td>13.3</td>
<td>0.263</td>
<td>0.17</td>
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<tr>
<td>PPA (%)</td>
<td>21.7</td>
<td>9.0</td>
<td>0.272</td>
<td>-0.25</td>
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<tr>
<td>AIx (%)</td>
<td>27.1</td>
<td>11.9</td>
<td>0.334</td>
<td>0.21</td>
</tr>
<tr>
<td>PP (mmHg)</td>
<td>66.6</td>
<td>17.9</td>
<td>0.000</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Wide variation in radial artery FF results in substantially different values of MP being calculated if a set formula such as diastolic + 33% of PP is used. The value of radial artery tonometry is that it provides an arithmetically averaged MP over the cardiac cycle, so that no fixed FF is applied. When the brachial waveform cannot be measured reliably, the radial artery is suitable for use of applanation tonometry to calculate MP in the upper limb.

DOES SELENIUM DEFICIENCY EXPOSE THE HYPERTENSIVE EFFECT OF AN OMEGA-3 FATTY ACID-DEFICIENT DIET?

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Increased oxidative stress has been suggested to contribute to hypertension. Although omega-3 (ω-3) fatty acid deficiency has been shown to increase oxidative stress, development of hypertension has been inconsistent. The aim of the present study was to determine whether elimination of the antioxidant selenium (Se) would lead to hypertension in animals maintained on aω-3 fatty acid deficient diet. At 7 weeks of age, male Sprague-Dawley rats (N=40) were divided into four groups and placed on semi-synthetic diets that contained identical amounts of protein (15%), from torula yeast 30% w/w, carbohydrate (53% w/w), fat (7% w/w), 4-hydroxyvitamin and minerals except for Se. The diets were either sufficient [ω-3 = 1.7; % cansa oil; ω-1:ω-6:ω-9 = 1:7:11] or deficient (ω-3 = 0.12; % cansa oil; ω-1:ω-6:ω-9 = 1:7:11). In groups maintained on an ω-3 fatty acid deficient diet, at 12 weeks of age, the average arterial pressure was 133±10 mm Hg, compared with 116±10 mm Hg in the ω-3 sufficient diet animals. Thereby, Se deficiency did not alter blood pressure in animals maintained on the ω-3 sufficient diet. The ω-3 deficient group showed higher plasma renin substrate levels in both ovarian reserve and age, systolic BP levels after initiation of such treatment were more important predictors of haematoma growth than baseline BP levels in acute ICH. BP levels in the first 24 hours were clearly associated with absolute haematoma growth: 1.1 ml (95% CI 0.2– 4.9 ml) for tertile groups defined by baseline BP levels of 170, 171–190, and 191 mm Hg (P<0.03 for trend).

ACUTE CARDIOVASCULAR RESPONSE TO STRESS: MODULATION BY THE RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM

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Chronic activation of the renin angiotensin system leads to hypertension, which may be partly neurogenically mediated. In support of this, we have shown that 2-week subcutaneous infusions of angiotensin II (AngII) lead to activation of specific hypothalamic nuclei known to be involved in regulating sympathetic activity, notably in response to stress. In the present study, we wished to determine whether the cardiovascular responses to acute and chronic stress of the adrenal and oxytocinergic systems were affected by chronic AngII treatment. To this end, we used conscious rabbits infused with low dose Ang II (10–30 ng/kg/min) for 5 weeks or by daily stress (airjet) for 1 week. A separate group received both treatments and a control group was included. We also determined the contribution of central and peripheral AT1 receptors in the response to stress using the AT1 antagonist Candesartan. All rabbits were instrumented with an intracerebroventricular (ICV) catheter and an electrode to measure renal sympathetic nerve activity (RSNA). RSNA responses to acute airjet stress were less in chronically stressed animals compared to control animals, presumably due to habituation to the stress. The responses to chronic daily stress were lower than to acute airjet stress. However, there was no effect of AngII treatment on the RSNA responses to acute airjet stress. Furthermore, the AT1 receptor antagonist candesartan (10nmol ICV) reduced RSNA response to stress in those animals receiving chronic stress treatments but had no effect on the RSNA response to stress in the control group or those given AngII alone. Our results show that increasing circulating AngII at levels that modestly increase blood pressure, prevents the habituation of sympathetic responses to stress through activation of central AT1 receptors.

EFFECTS OF BLOOD PRESSURE LEVELS AT PRESENTATION AND ACHIEVED IN THE FIRST 24 HOURS ON HAEMATOMA GROWTH IN ACUTE INTRACEREBRAL HAEMORRHAGE: THE INTERACT TRIAL

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Early elevation of blood pressure (BP) is common and predicts haematoma growth and other adverse outcomes in acute intracerebral haemorrhage (ICH). However, uncertainty persists as to whether there are beneficial effects of early BP lowering treatment. We used data from the Intensive Blood Pressure Reduction in Acute Cerebral Haemorrhage Trial (INTERACT) pilot phase to determine the importance of systolic BP levels at presentation and achieved in the first 24 hours, as predictors of absolute and relative haematoma growth in ICH. INTERACT included 404 patients with elevated systolic BP (150–220 mmHg) within 6 hours of CT-confirmed ICH. Digital images of baseline and repeat CT (24:±3 hours) were performed using standardised techniques and analysis procedures. The association of BP levels on absolute and proportional changes in haematoma volumes were assessed by an analysis of covariance (ANCOVA) with age, sex, log of baseline haematoma volume, haematoma location, time from CT to ICH onset, and analysed centrally. The effects of BP levels on absolute and proportional changes in haematoma volumes were assessed by an analysis of covariance (ANCOVA) with age, sex, log of baseline haematoma volume, haematoma location, time from CT to ICH onset, and randomised treatment, as covariates. Overall, 346 patients with 2 CT scans were available for analyses. Absolute growth in haematoma volume increased with higher baseline BP levels: 2.1 ml (95% CI 0.6–4.6 ml) vs 2.3 ml (95% CI 0.2–4.9 ml) and 3.6 ml (95% CI 1.0–6.3 ml) for tertile groups defined by baseline BP levels of ≤170, 171–190, and ≥191 mmHg. However, these associations were not significant (P=0.27 for trend). In contrast, mean BP levels in the first 24 hours were clearly associated with absolute haematoma growth: 1.1 ml (95% CI 1.4–3.7 ml), 3.0 ml (95% CI 0.2–5.7 ml) and 4.2 ml (95% CI 1.7–7.2 ml) for tertile groups defined by achieved BP levels of ≤143, 144–158, and ≥159 mmHg (P=0.03 for trend). Likewise, relative increase in haematoma volume was significantly associated with mean systolic BP levels during the first 24 hours (P=0.03 for trend), but not with baseline BP levels (P=0.12 for trend). These results suggest beneficial effects of early BP lowering as achieved BP levels after initiation of such treatment were more important predictors of haematoma growth than baseline BP levels in acute ICH.
ENDOTHELIAL DYSFUNCTION IN AGED SPONTANEOUSLY HYPERTENSIVE RATS IS REVERSED BY NADPH OXIDASE INHIBITION

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Arterial hypertension is associated with increased formation of reactive oxygen species (ROS), ROS reacts with NO, reducing the bioavailability of this vasorelaxant. The resultant impaired endothelium-mediated vasorelaxation, i.e. endothelial dysfunction is a hallmark of cardiovascular diseases such as hypertension. NADPH oxidases are enzymes that solely produce ROS, and hence represent a novel target for the treatment of hypertension. NOX1 may thus represent a novel target for the treatment of hypertension. Importantly, the ectopic expression of NOX1 in aortae as is expression of NOX1 and NOX2. In conclusion, ROS formation is increased in aged SHR and WKY aortae. In conclusion, ROS formation is increased in aged SHR and WKY aortae. In conclusion, ROS formation is increased in aged SHR and WKY aortae. In conclusion, ROS formation is increased in aged SHR and WKY aortae.
**CVD IN INDIGENOUS AUSTRALIANS: OPPORTUNITIES FOR IMPROVING OUTCOMES ACROSS THE CONTINuum OF CARE**

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Whilst recent political and health system reforms has focused on ‘closing the gap’ in health status for Indigenous Australians, less attention has been afforded to outlining the specific activities which are most likely to reduce this gap. Amongst a long list of health issues driving the disparity experienced by Indigenous Australians, Cardiovascular Disease (CVD) remains the primary target. They are the principal cause of death and of excess death among Indigenous people in Australia, and account for almost one-third of the life expectancy gap. More particular is the significant disparity at younger ages, with both Aboriginal men and women up to ten times more likely to die from CVD between the ages of 25–54 years. In terms of explaining these gaps, most attention has focused on the higher burden of traditional risk factors experienced by Indigenous people. Far less attention has focused on the quality and outcomes of health system performance in explaining these differentials. What limited information exists suggests that there are significant barriers to necessary care for Indigenous Australians, in rural/remote areas and in metropolitan settings. National data has identified poor outcomes and use under necessary procedures for Indigenous people experiencing acute events.

This presentation reports on extensive qualitative and quantitative clinical research of CVD in the NT, focused on the patterns, burdens, provision of care, experience of services, adverse outcomes and their determinants, and opportunities for reform within the management of CVD among Aboriginal Australians.

**INCREASED TISSUE KALLIKREIN EXPRESSION IN HUMAN TYPE 2 DIABETES**

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The kallikrein kinin system contributes to inflammation and organ-protection. Loss of function mutation of the tissue kallikrein gene is associated with arterial dysfunction in humans and gene knockout studies show an essential role for tissue kallikrein in arterial function, ischaemic preconditioning, cardiac remodeling and survival after myocardial infarction. Moreover, kinin peptides mediate in part the benefits of angiotensin converting enzyme inhibitor and angiotensin II type 1 receptor blocker therapies. To investigate the expression of the kallikrein kinin system in human type 2 diabetes mellitus we measured circulating levels of bradykinin and kallidin peptides, high and low molecular weight kininogens, plasma and tissue kallikrein, and kallistatin in non-diabetic and diabetic patients before coronary artery bypass grafts. Plasma levels of tissue kallikrein were approximately 62% higher in diabetic than non-diabetic patients (P<0.015), whereas there were no differences in circulating levels of bradykinin and kallidin peptides, high and low molecular weight kininogens, plasma and tissue kallikrein, and kallistatin in non-diabetic and diabetic patients before coronary artery bypass graft surgery. Further investigations of the kallikrein kinin system are needed to confirm and understand the role of the kallikrein kinin system in the control of blood pressure in type 2 diabetic patients.

**COMPARISON OF VARIOUS ANTHROPOMETRIC INDICES AS PREDICTORS OF CARDIOVASCULAR DISEASE IN PATIENTS WITH TYPE 2 DIABETES**

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Aims: The aim of this study was to compare the strength of associations and discrimination capability of body mass index (BMI), waist circumference (WC) and waist-to-hip ratio (WHR) to the risk of cardiovascular disease and death in patients with type-2 diabetes participating in ADVANCE (Action in Diabetes and Vascular Disease: Preterax and Diamicron MR Controlled Evaluation).

Methods and results: 11,140 men and women were followed for a mean of 4.8 years. Cox proportional hazard models were used to determine the hazard ratios (HR) and 95% confidence intervals (95% CI) for the risk of cardiovascular disease and death, associated with one standard deviation (SD) increase in baseline BMI, WC and WHR. After adjustment, HR (95% CI) for WC were 1.10 (1.03–1.18) for cardiovascular events, 1.13 (1.03–1.24) for coronary events, and 1.69 (1.58–1.81) for cardiac deaths. Estimates for WHR were 1.12 (1.05–1.19), 1.17 (1.08–1.28) and 1.19 (1.09–1.31). BMI was not significantly associated with the risk of any of these outcomes. While the receiver operating characteristics curve could not differentiate between the predictive capacity of these anthropometric variables (p-values > 0.7), the more frequent use of any of these outcomes with WHR indicates increased discrimination improvement statistic showed an enhancement in the discrimination capabilities of models using WHR for cardiovascular outcomes, except for cerebrovascular events.

**THE ROLE OF THE MEDIAL NUCLEUS OF THE AMYGDALA IN SCHLAGER (BPH/2J) GENETICALLY HYPERTENSIVE MICE**

PJ Davern, T-P Nguyen-Huu, L La Greca, GA Head, Baker IDI Heart & Diabetes Institute, Melbourne

The amygdala located in the limbic system has long been associated with mediating the emotional and hormonal response to stress. While there has been much focus on the role of the central nucleus of the amygdala (CeM), there is an increasing awareness that the medial nucleus of the amygdala (MeM) is a major region activated to specific “stressful” stimuli. Studies in rats have shown that the MeM retains the ability to activate the sympathetic nervous system of obese mice, even though they are otherwise resistant to leptin, therefore leptin resistance is not global, rather it is selective. Hyperleptinemic mice are hypertensive and tachycardiac, conversely leptin deficient mice are Bradytachycardiac and hypertensive. In response to leptin, ob/ob leptin resistant mice still show rapid elevation of neurons in the dorsomedial hypothalamus, and rapid increases in increase adipocyte temperature. The temperature increases and neuronal activation are blocked by pretreatment of the dorsomedial hypothalamic nucleus with a leptin antagonist. Here we describe the effects of chronic leptin treatment on cardiovascular tone in lean mice, and of leptin antagonism on cardiovascular tone in obese mice.

**DISTRIBUTION OF DIFFERENTIAL GENE EXPRESSION IN GENETICALLY HYPERTENSIVE MICE**

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The aim of this study was to identify the genes whose expression differs between the Schlag high blood pressure (BPH/2J) and normal blood pressure (BPN/3J) strains of mice. Recent data points to an important role for the hypothalamus in the cause of hypertension in BPH/2J mice. Preliminary microarray results using the latest Affymetrix GeneChips mouse arrays and RNA extraction from the hypothalami of 3 BPH/2J and 2 BPN/3J mice aged 16 and 17 week old, respectively, led to the identification of 96 genes (173 probes) whose expression differed between each strain, adjusted for a false discovery rate of 0.05 (adjusted p-values ranging from 0.0026 to 0.049). Amongst these genes were ones implicated previously in hypertension, such as the aminolevulinate gene (ALAD), coiled-coil domain containing 157 gene (CDDC157), and hydroxysterolglarginase hexoside synthase 1 gene (HPGD). Other interesting genes, never related to hypertension before, such as the collagen triple helix repeat containing 1 gene (CHTRC1), DEAD (Asp-Glu-Ala-Asp) box polypeptide 4 gene (DDX4), growth arrest specific 5 gene (GASS), glycrosphosphodiester phosphodiesterase domain containing 3 gene (GDPS3), halocid dehalogenase-like hydrolase maintaining 3 gene (HIDH3), phosphofructokinase A gene (PFKAB), and processing precursor 4, ribonuclease P/MRP subunit (Saccharomyces cerevisiae) (P04), showed differential expression between the two strains. A functional annotation clustering with gene ontology enrichment identified groups of genes involved in nucleosome activity, translation and post-transcriptional protein modification, ion binding, hydroxide activity, organelle organization and biogenesis, as well as other functions. In conclusion, our preliminary array data has identified novel genes whose hypothalamic expression is altered in hypertension. Some of these may be causative and others include changes to maintain or counteract the hypertensive state.

**DOES HYPERLEPTINEMIA CAUSE HYPERTENSION IN OBESITY?**

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Obesity rates continue to rise, presenting greater risks of diseases that are co-morbid with obesity. We set out to determine the contribution of obesity and leptin to the risk of diabetes and cardiovascular diseases. We have previously described the phenomenon of leptin-resistance, where obese mice no longer lose weight in response to the adipocyte hormone leptin. We have discovered that leptin retains the ability to activate the sympathetic nervous system of obese mice, even though they are otherwise resistant to leptin, therefore leptin resistance is not global, rather it is selective. Hyperleptinemic mice are hypertensive and tachycardiac, conversely leptin deficient mice are Bradytachycardiac and hypertensive. In response to leptin, leptin resistant mice still show rapid activation of neurons in the dorsomedial hypothalamus, and rapid increases in increase adipocyte temperature. The temperature increases and neuronal activation are blocked by pretreatment of the dorsomedial hypothalamic nucleus with a leptin antagonist. Here we describe the effects of chronic leptin treatment on cardiovascular tone in lean mice, and of leptin antagonism on cardiovascular tone in obese mice.
associated with night-time active periods in the Schlafter (BPV/LJ) hypertensive mice leading to hypertrophy and an exaggerated circadian day-night difference in BP.

TOPOGRAPHY AND PROJECTION OF BLOOD PRESSURE SENSITIVE NEURONS IN THE LATERAL PARABRACHIAL NUCLEUS

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The lateral parabrachial nucleus (LPBN) is increasingly viewed as a critical integrative site for the transfer of visceral cardiovascular information from the brainstem to a number of regions in the hypothalamus. The goals of this study were to examine the topography and relationship between BP sensitive neurons located in LPB subnuclei and their projections to the median preoptic nucleus (MnPO) and central nucleus of the amygdala (CeAm). Retrogradely transported neuronal tracer was injected into either the MnPO (n = 8) or the CeAm (n = 8) and rats underwent a 1 wk recovery period. C-Fos immunohistochemistry was used to detect neurons in the LPBN activated in response to iv infusion of phenylephrine hydrochloride (10 μg/kg/min; n = 8) or sc injection of diazoxide (45 mg/kg; n = 8) in experimental groups; or iv infusion or sc injection of isotonic saline (n = 8) in control groups. Neuronal activation in rats infused with iv phenylephrine was greater in the central-dorsal central, and external, and superior lateral subnuclei (P < 0.001) compared with vehicle infused controls. The findings demonstrate that efferent projections arising from neurons in the central (43%), dorsal (31%) and dorsal part of the external (60%) lateral parabrachial nucleus innervating the MnPO also respond to an increase in BP. Alternatively, 63% of neurons located in the central part of the external lateral FBN that innervates the MnPO were sensitive to a rise in BP. Although neuronal activation in rats with reduced arterial pressure induced by sc injections of diazoxide was greater in the central LPB and central part of the external LPB subnuclei compared with saline infused controls, these cells were not retrogradely labelled from either the MnPO or the CeAm. This is the first demonstration of direct neuronal projections arising from pressor responsive neurons in discrete LPB subnuclei innervating neurons in the MnPO and CeAm. Although, there was no evidence for ascending projections from neurons activated by hypotension terminating in either the MnPO or CeAm, this study does provide topographic evidence for separate populations of neurons in the LPBN responding to elevated and reduced BP.

EXPRESSION OF RENALASE, A NOVEL MONOAMINE OXIDASE, IN ADIPOSE TISSUE

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The prevalence of obesity is increasingly recognised as important risk factors for the development of a number of cardiovascular related conditions including hypertension. The precise mechanisms linking obesity to hypertension are still only incompletely understood. One of the mechanisms governing this increased risk involves activation of the sympathetic nervous system. Up till recently membrane bound monoamine oxidases (MAO) A/B and semicarbazide-sensitive amine oxidase have been responsible for the breakdown of catecholamines in human tissues and in circulation. Renalase, a novel soluble monoamine oxidase and activity has been identified in the human kidney and heart. Given that adipose MAO A/B expression and activity in obesity is altered, in the present study we aimed to determine whether renalase is synthesised by adipocytes and whether its expression is altered with weight loss in patients with the metabolic syndrome.

Twenty-three subjects with metabolic syndrome participated in this study. To assess the effects of weight loss on sympathetic activity they were randomised into a diet, diet and exercise or control (no treatment) group. Indices of sympathetic activity included whole body and muscle sympathetic nerve activity. Renalase was readily detectable in all adipose tissues and in circulation. Recently, a novel soluble monoamine oxidase, renalase, has been identified in the human kidney and heart. Given that adipose MAO A/B expression and activity in obesity is altered, in the present study we aimed to determine whether renalase is synthesised by adipocytes and whether its expression is altered with weight loss in patients with the metabolic syndrome.

HYPERLEPTINEMIA CONTRIBUTES TO HYPERTENSION BY SYMPATHOEXITATION IN OBESITY

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Leptin is a hormone released from fat tissue which promotes weight loss by reducing appetite and by increasing energy expenditure through activation of the sympathetic outflow to thermogenic tissues. In obese states there is a resistance to the metabolic (satiation and weight-reducing) actions of leptin. Leptin resistance appears to be selective, and it is possible that leptin activates some neurons that regulate the sympathetic nervous system (SNS) in obesity. To evaluate the hypothesis that stimulation of SNS by leptin causes an increase in intercapsular temperature (IT), blood pressure (BP), and heart rate (HR), mice were fed a high fat diet (45 % fat). Diet induced obese (DIO) mice were compared with mice on a control diet (12% fat) and with genetically obese leptin deficient (ob/ob) mice. Sympathetic outflow to brown adipose tissue (BAT), skeletal muscle, heart, and carotid body motor activity was measured. Activity was 2.5 fold higher in DIO than control mice (A 0.6 °C, P<0.001). On the contrary, ob/ob mice showed lower IT than controls (P<0.001). LA was similar in DIO and controls, but ob/ob mice were hyperactive. DIO mice had a significant increase in IT after peripheral or central leptin administration, despite no difference in caloric intake, showing that the thermogenic response to leptin was intact. Leptin also induced p-Stat3 expression (a well-known mediator of leptin activation) in dorsal medial (DMH) but not in arcuate nucleus of the hypothalamus of DIO mice. Leptin given directly into the DMD significantly increased IT in all groups. Mean arterial pressure (MAP), measured by radio-transmitters was higher in lean compared with lean controls (115 ±1.7 vs 103.3 ±1.5 mmHg, P<0.001) as well as systolic BP (128.1 ±2.5 vs 117.5 ±1.9, P<0.01), diastolic BP (100.7 ±1.9 vs 93.3 ±1.3, P<0.001) and HR (550.4 ±11.5 vs 475.9 ±7.2, P<0.001). Chronic peripheral leptin infusion increased systolic BP in lean but not DIO mice (P<0.05). Leptin levels strongly correlated with MAP in DIO mice (r2=0.99, P<0.001). These results suggest that DIO mice are resistant to leptin’s suppressive effect on appetite, but not to the sympathetic regulation of BP and BAT. This strongly suggests that DMO may mediate the sympathetic responses during hyperleptinemia.

FLOW AND ENERGY LOSS IN SACULARANEURYSMS ARISING FROM STRAIGHT AND CURVED PARENT ARTERIES - HYDRAUODYNAMIC ANALYSIS FOR ASSESSING RISK OF RUPTURE

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Sacculaneurysms are the most common type of cerebral aneurysms with 50% mortality due to rupture. Although risk of rupture is conventionally associated with aneurysm size, development of imaging technology and computational fluid dynamic (CFD) analysis has enhanced the scope of investigations of rupture beyond aneurysm size and shape. The aim of this study was to investigate the effect of parent artery geometry on intra-aneurysm flow patterns and energy loss (EL). CFD analysis was performed on finite volume models of sacularaneurysms fed with curved or straight parent arteries ranging from 4, 5 and 6 mm diameter; aneurysm depth 6–10 mm; aneurysm neck width 4.8 mm. Calculations were performed with pulsatile and steady flow rate (300 ml/min) at inlet and outlet. EL was calculated as the energy difference between parent artery inlet and outlet for each parent artery diameter of straight and curved parent artery models. The result indicated that EL was not dependent on aspect ratio (depth/neck width) over the range 1.25–2.1. However, appearance of secondary flow in aneurysms fed by curved parent arteries increased the magnitude of EL two fold compared to the flow fed by straight parent arteries. A small (4 mm) curved parent artery resulted in the highest relative EL and flow rate inside the aneurysm for all aspect ratios. In conclusion CFD analysis shows that intra-aneurysm flow patterns and energy loss due to the saccular malformation are affected by the geometry of parent arteries. Since flow changes can affect both growth and rupture of aneurysm, this analysis can be used to differentiate aneurysms of similar size but with different risk of rupture.

HYDROGEN SULPHIDE INHIBITS PLATELET DENSE GRANULE SECRETION BUT NOT PLATELET ACTIVATION

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Hydrogen sulphide is a gaseous mediator endogenously generated in the heart and blood vessels. It has been shown to cause vasorelaxation and plays a role in cardioprotection from ischaemic injury. Recent studies have shown that extra-physiological but sub-toxic doses of hydrogen sulphide may inhibit platelet aggregation in vitro. We sought to elucidate the mechanism by which such inhibition might occur. Platelet rich plasma was obtained from normal healthy volunteers and incubated with 10 μM hydrogen sulphide or pH matched vehicle control for 20 seconds prior to initiation of platelet activation and aggregation with chemical agonists. While samples treated with hydrogen sulphide exhibited normal shape change and initiated primary aggregation, secondary platelet aggregation was inhibited by hydrogen sulphide versus vehicle control in response to 0.5 μM arachidonic acid (5.6% vs 81.4%, P<0.01), 2 μM collagen (28% vs 82%, P<0.01), 0.5 μM of thromboxane mimetic U46619 (38.4% vs 67.7%, P<0.01) and 7 μM adrenaline (19.6% vs 76.5%, P<0.01). In hydrogen sulphide treated blood, marked disaggregation was observed following normal initial shape change and primary aggregation in response to 2 μM adenosine diphosphate (20.2% vs 72.7%, P<0.01) indicating that while platelet activation was normal, stability of the platelet aggregates was disturbed by hydrogen sulphide. ATP release measured by luciferase was completely inhibited by hydrogen sulphide for all chemical agonists tested, while P-selectin expression, measured by flow cytometry, was unaffected and the structure and function of the platelet fibrinogen receptor GPIIb-IIIa, as reported by PAC1 binding, was also unaffected. This suggests that hydrogen sulphide inhibits platelet contraction and secondary aggregation leading to reduced stability of platelet aggregates through prevention of dense granule release and therefore secretion of important signalling molecules such as ATP, ADP, adrenaline, serotonin and thromboxane A2. However, primary platelet activation, initial aggregation, shape change and release of alpha granules containing adhesion molecules such as P-selectin are unaffected. Therefore hydrogen sulphide may have significant therapeutic benefit by preventing propagation of secondary aggregation through primary platelet activation and aggregation in response to injury and exposure to sub-endothelial collagen, thus potentially ameliorating the increased bleeding risk associated with other forms of antithrombotic therapy.
HOMOCYSTINE AND DNA DAMAGE GENETIC POLYMORPHISMS ARE ASSOCIATED WITH HYPERTENSION IN PREGNANCY

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Hypertensive disorders of pregnancy are a major cause of both maternal and fetal morbidity and mortality. Preeclampsia affects 5–8% of all pregnancies, while 10–20% of mothers will have a hypertensive disorder during pregnancy. Previous studies have associated the C677T methylenetetrahydrofolate reductase (MTHFR) gene polymorphism and plasma homocysteine concentration with hypertension, preeclampsia and DNA damage. This was a prospective study involving 1169 nulliparous pregnant couples. Samples and patient information were collected by SCOPE research midwives. Genotyping was performed by Sequenom MassARRAY. Non-European couples and those who required fertility treatment were excluded from analyses. Pregnancy outcomes were strictly classified: PE (n = 71), PE + SGA (n = 20), controls (n = 408). Chi Square and univariate ANOVA with post-hoc analyses were performed. Both paternal and neonatal methionine synthase (MTHFS) 275G6 alleles were associated with PE + SGA compared to controls (P = 0.043, resp). Maternal methionine synthase reductase (MTRR) G1299A genotype was associated with healthy pregnancies (P = 0.030). Neonatal NAT110T allele corrected for age and BMI was associated with maternal mean arterial pressure and sBP (P = 0.011, P = 0.001). MTR and MTRR work together to catalyse the methylation of homocysteine to methionine. Polymorphisms within these genes slow enzyme activity increasing plasma homocysteine leading to vascular damage and possibly increased blood pressure. Furthermore, formation of methionine through this pathway is important for healthy placental and fetal development. The NAT1 enzyme catalyses the N-acetylation of aromatic amines and hydroxycinnamic acid. Polymorphisms in NAT110 reduce detoxification potential and have been associated with increased DNA damage and various types of cancer. In this study we have detected an association with neonatal NAT110 polymorphisms and maternal mean arterial pressure and sBP at 15 weeks gestation. These indicate that fetal/paternal metabolic enzymes may contribute to raised maternal blood pressure and that those associated with increased homocysteine may link with preeclampsia, a common hypertensive disorder in pregnancy.

ASSESSMENT OF CARDIAC FUNCTION WITH TRANS-THORACIC ECHOCARDIOGRAPHY IN ADULT VITAMIN D DEFICIENT RATS

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Vitamin D is a fat-soluble vitamin which is essential in bone metabolism, cell growth, differentiation and regulation of the immune system. The duration of sunlight is the major source of vitamin D. There is an increasing prevalence of vitamin D deficiency in many populations world-wide, resulting from both inadequate exposure to ultraviolet light and diet intake. It is well known that vitamin D deficiency is associated with heart disease. We have recently demonstrated in the rat heart that vitamin D deficiency leads to cardiac hypertrophy and vulnerability to ischemia later in life, and female offspring appear to be most vulnerable. The aim of the present study was to investigate the effect of vitamin D deficiency on cardiac function, using echocardiography, in 14 week old adult rats. Four week old Sprague-Dawley female rats were fed either a vitamin D deplete or vitamin D replete (control) diet for 6 weeks prior to pregnancy, during pregnancy and throughout lactation. Offspring remained on their respective diets until adulthood. Hearts of 16 week old male and female offspring (n = 8/group) were isolated on a Langendorff apparatus. Basal heart rate (HR), coronary flow, rate of contraction (+dp/dt) and relaxation (-dp/dt) and response to isoprenaline were recorded. The hearts were then subjected to 20 minutes ischemia and 1½ hours reperfusion. At the end of the reperfusion period the left ventricle was sliced and incubated in 1% 2, 3, 5 triphenyl tetrazolium solution (TTZ), to determine infarct area using computerized planimetry. Basal cardiac function (HR, +dp/dt, -dp/dt) was not different between groups. Basal coronary flow was lower in hearts of vitamin D deficient rats. The isoprenaline-induced increase in HR tended to be greater in vitamin D deficient males (P = 0.06), but there was no differences in contractile function between groups. After 55 minutes reperfusion, HR had declined by 30% of that before ischemia in both males and females, with HR being higher in vitamin D deficient males compared with control males. Infarct area was 2 fold greater in vitamin D deficient hearts of both males and females (P = 0.006 & P = 0.03, respectively). Basal and stimulated heart function was not altered, although coronary flow was significantly reduced in vitamin D deficient rats. In conclusion, the hearts of vitamin D deficient rats are particularly susceptible to ischemia/reperfusion injury. Dysregulation of coronary flow and the extent of vascularisation may be factors which contribute to the increased susceptibility to ischemia/reperfusion injury.

THE THIAZIDE-SENSITIVE NA-CL COTRANSPORTER IN THE DISTAL NEPHRON (NCT) IS REGULATED BY BOTH KINASES AND PHOSPHATASES

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Dissecting the molecular regulation of renal sodium transport by the thiazide-sensitive NCT has provided important insights into the mechanisms underlying hypertension. NCT regulation is complex, including by a scaffold, CIB1, including SKAP 1 and WNK kinases. Inhibitors of such dynamic regulation must also involve phosphatases. Two distinct phosphorylation controlled regulatory pathways for NCT exist; Type 1 mediated by WNK3/4 affecting trafficking to the surface membrane and Type 2 involving modulation of intrinsic transporter kinetics by phosphorylation of β-terminus and TES. Inhibition of WNK kinases has been hampered partly by steric constraints of key residues blocking entry of small molecule inhibitors to the active site of the enzyme.

To address this we used site mutation to produce constructs of NCT T562A, kinase-dead PP4 and WNK4/2T61A. Xenopus oocytes were then injected with cRNA from these constructs and NCT expression followed using 22Na flux and confocal microscopy. PP4 expression inhibited NCT activity by 68% without affecting surface membrane expression. The effect was specific to PP4 and dependent on both its phosphorylation activity and NCT-T562 to accept the phosphate. Phosphatases did not affect WNK4 regulation of NCT membrane trafficking. In addition, site mutation of WNK4 T261A enhanced fifteen fold its susceptibility to the kinase inhibitors SB202190 and SB203580.

Thus NCT activity but not trafficking is regulated by PP4 with clear implications for blood pressure control. Furthermore, single site mutation of WNK4 increases significantly its sensitivity to established kinase inhibitors increasing the prospects for pharmacological investigation of this pathway in vivo.

NON-INVASIVE ASSESSMENT OF CEREBROSPINAL FLUID PRESSURE BY MEANS OF SPONTANEOUS RETINAL VENOUS PULSATIONS: A MODELING STUDY

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Spontaneous pulsations of retinal venous pulses have been shown to arise because of pressure gradient which exists between the intraocular and intracranial compartments. Invasive studies have demonstrated that the higher systole to diastole ratio of pressure in the intracranial space compared that in the intraocular space is the origin of this pressure gradient. Intraocular hypertension, cerebrospinal fluid pressure (CSFp) pulsatility increases align with mean CSF pressure and as it approaches the intracranial pulse pressure (IPP) the intravascular pressure gradient decreases respectively, leading to cessation of pulsations. Characterizing interaction between ocular and intracranial space pressure in humans involve difficult and limited surgical procedures. Therefore in this study we developed a model to characterize the pressure distribution between the intra and extra ocular space. Electrical circuit elements are used to construct the model, which incorporates both vascular and CSF resistance and compliance. The model is applied to extract an index between the retinal venous pulsations and CSF pressure. Intraocular hypertension is simulated by increasing the CSF outflow resistance or a decrease in intracranial compliance. The retinal venous pressure is obtaining as a function of the difference between IPP and CSFp. Simulation results show an increase in CSF outflow resistance will increase the CSF pressure which in return decreases the retinal venous pulsations. This interaction could be described as: RVp = -0.109RVp + 1.616RVp - 2.965.

Which RVp is the Retinal Venous Pulsatility. Results also show a decrease in IPP will decrease the pulsatility of the retinal veins verifying an equilibrium is present between pressure in the intra and extra ocular space.

The model presented in this study describes the interaction between the retinal venous pulsations and cerebrospinal fluid pressure. It explains the physiological structure governing the intraocular and intracranial space. The model may allow a non-invasive approach to cerebrospinal fluid pressure estimation.
NEUROPEPTIDE CODING OF ADRERNALLY PROJECTING SYMPATHETIC PREGANGLIONIC NEURONS

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Different physiological stimuli selectively evoke release of noradrenaline (NA) or adrenaline (Ad) from the adrenal medulla. Therefore NA and Ad chromaffin cells are innervated by different populations of sympathetic preganglionic neurons (SPN). How these populations differ functionally and structurally has not been considered although it is suggested that NA innervating the adrenal medulla are chemically coded. The neuropeptides pituitary adenylate cyclase activating polypeptide (PACAP) and enkephalin have been proposed as neuromodulators mediating catecholamine secretion from the adrenal medulla based on immunohistochemical localization of terminals of preganglionic sympathetic fibers and mRNA. We aimed to determine the distribution and proportion of SPN that contain PACAP or preenkephalin (PPE) mRNA, 2) to determine the distribution and proportion of adrenally projecting SPN that contain PACAP or PPE mRNA and, 3) in light of the proposed sensory innervation of the adrenal medulla, to determine whether sensory neurons in the different sensory ganglia (DRG) that project to the adrenal medulla contain PACAP or PPE mRNA. Digoxigenin-labeled riboprobes encoding PACAP and PPE were synthesized. Following anaesthesia and perfusion, spinal cord sections from male Sprague Dawley rats were processed for in situ hybridization (ISH) for PACAP and PPE combined with immunohistochemistry (IHC). Study 1: PACAP/PPE ISH combined with IHC for vesicular acetylcholine transporter (vAChT). Study 2: PACAP/PPE ISH combined with IHC for cholera toxin B (CTB-ir), identifying adrenally projecting neurons after CTB injections into the adrenal medulla. We found in total SPN that few were PACAP (+4.2%) whilst the majority were PPE (+80.3%). In adrenally projecting SPN, 47.3% were PPE (+), whilst 97.3% ± 2% were PACAP (+). In adrenally projecting DRG ±1% were PPE (+), whereas 74% ±12% were PACAP (+). In summary, we found that the majority of total SPN contain PACAP mRNA while few SPN contain PPE mRNA. We have established that all adrenally projecting SPN contain PACAP and 50% of adrenally projecting DRG contain PACAP. Given the proposed sensory innervation that the adrenal medulla receives sensory innervation that is largely PACAPergic. This may provide a neurochemical basis for differential control of sympathetic outflow to the adrenal medulla.

SYNTHESIS OF RENALASE, A POTENTIAL NOVEL MONOAMINE OXIDASE, IS NOT LIMITED TO RENAL AND CARDIC TISSUE

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Twenty-four hour ambulatory blood pressure (ABP) thresholds exist for the diagnosis of hypertension but not for other blood pressure (BP) thresholds used in the management of hypertension. We have previously reported ABP equivalents for both hypertension diagnosis and management thresholds using clinic BP measured by trained staff. However predicted ABP values were considerably higher than those reported by the landmark PAMELA study, which may be due to physicians recording clinic BP values. In the present study we have compared ABP equivalents for seated clinic systolic BP (SBP) and diastolic BP (DBP) measured by trained staff or by physician. ABP data was collected from 10 centres across six Australian States (n=8,386), Ordinary least product (OLP) linear regression analysis was used to relate predicted clinic ABP and ABP values. Subjects were on average 56 years old (54% female), with clinic SBP/DBP of 142/82 mm Hg. Regression coefficient vs clinic blood pressure predicted that daytime ABP equivalents were 4/3 mmHg lower at the 140/90 mmHg threshold (lower limit of grade 1 hypertension), 2/2 mmHg lower at 130/80 mmHg (upper limit with one condition), and 0/0 mmHg lower at 125/75 mmHg. Equivalents were 2/3 mmHg lower for females than males and 2.4 mmHg lower in older subjects (SBP only below 140 mmHg) for both sexes. Staff measured clinic BP was 8/7 mmHg lower than clinic BP measured by physicians and predicted ABP values correspondingly higher. The latter closely matched the findings of the PAMELA study. Our study suggests that physician measurement of clinic BP may inappropriately modify the estimates of ABP thresholds for treatment. Furthermore, clinic BP measured by trained staff was similar to daytime ABP. These findings suggest that our initial framework of ABP equivalents based on staff clinic measurements is valid and appropriate for both the diagnosis and management of hypertension.

ASSOCIATION OF HYPOTHALAMIC PITUITARY AXIS GENES WITH LONGITUDINAL CHILDHOOD SYSTOLIC BLOOD PRESSURE

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Childhood blood pressure is predictive of adult hypertension. Genes of the hypothalamic axis may affect blood pressure control. In adult studies, association studies have shown that NR3C2, encoding the glucocorticoid receptor, is associated with both systolic and diastolic blood pressure. In children, a recent genome-wide association study identified 66 SNPs associated with blood pressure (BP), of which 27 were in the glucocorticoid receptor gene, NR3C2. In this study, we investigated genetic variants in NR3C2 and other genes of the hypothalamic axis for association with both systolic and diastolic BP in children, and investigated sex differences. This study included a cohort of 2,383 children (age 4–12 years) from the Hordaland Health Study in Norway. We genotyped 66 SNPs in NR3C2 and 15 SNPs in 12 other genes of the hypothalamic axis, using a custom array. Out of the 81 SNPs, 66 were genotyped successfully. Twenty SNPs were associated with systolic BP and 62 with diastolic BP. SNP rs17194273 in NR3C2 was associated with both systolic (p=0.002) and diastolic (p=0.003) BP. After false discovery rate correction, rs17194273 was the only SNP associated with both systolic and diastolic BP. After stratification by sex, the association was stronger in girls than boys for systolic BP (p=0.009) and diastolic BP (p=0.042). These findings suggest that genetic variants in NR3C2 are associated with childhood blood pressure.
genes of the hypothalamic axis, in particular NR3C2, GHR1R, GHRFR, PCSK2, leptin, leptin receptor and PPARα may have a role in childhood blood pressure control. These results are currently being replicated in other pregnancy cohorts.

A CROSS-SECTIONAL STUDY ASSESSING THE RELATIONSHIP BETWEEN DIETARY SODIUM AND BLOOD PRESSURE IN AN AUSTRALIAN POPULATION SAMPLE

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Excess sodium consumed throughout life contributes to the age-related rise in blood pressure (BP). Reducing dietary sodium or the dietary sodium to potassium ratio lowers BP. The relationship between dietary sodium and potassium intake and BP within an Australian population group has not previously been assessed. The aim of this study was to assess the relationship between dietary sodium and potassium intake and blood pressure in an Australian population sample, using the gold standard measurement of 24 hr urine excretion. A cross-sectional study was conducted using participants enrolled in the Melbourne Collaborative Cohort study. Daily intakes of sodium and potassium were measured from 24 hr urine samples provided by participants (2007/08). BP was assessed under standard conditions in a subgroup of this population. The mean age of participants (men n = 376, women n = 408) was 64.0 (6.3) (SD) years. For men and women respectively, the mean urinary sodium was 178.4 (66.6) and 133.7 (51) mmol/day (approximately 10 g and 8 g salt/day), mean urinary potassium was 88.1 (30.7) and 71.0 (23.9) mmol/day and the sodium to potassium (Na/K) ratio was 2.16 (0.88) and 1.93 (0.74) day. Only 21% of participants met the recommended intake of sodium (<100 mmol/day). In the 584 participants who provided blood pressure measurements, sodium and the Na/K ratio were both predictors systolic BP (age and sex adjusted): β coefficient (se) β = −0.34 (0.01) P < 0.002, β = −2.34 (0.89) P = 0.004, respectively. The Na/K ratio was also a significant predictor of diastolic BP (age and sex adjusted): β = −0.59 (0.54) P = 0.01, but sodium alone was not a significant predictor of diastolic BP (β = 0.01 (0.01) P = 0.149). After the addition of body mass index (kg/m²) to the model, sodium and the Na/K ratio remained significant predictors for systolic BP (P = 0.013, P = 0.013), but not for diastolic BP. In conclusion, following pretreatment with PACAP(6-38), PACAP-38 caused significant changes in MAP and sSNA (%). Following pretreatment with PACAP(6-38), a specific PAC1 receptor antagonist, the change in MAP. Additionally, it is unknown if PACAP is involved in either the tonic or reflex change in variables during dark cycle light. The diurnal range in BP and heart rate were 134% and 68% greater respectively in BPH compared to BPH normotensive mice (P < 0.001). However, the rate of rise in BP from baseline was significantly different between the two strains (2.7 ± 0.02 vs 8.4 ± 0.19 mmHg/h). Thus the duration of the surge in BP was markedly longer in the BPH hypertensive animals (3.2 ± 0.5 vs 1.8 ± 0.4 hr; P = 0.02) but the midpoint of the rise was similar. Comparison with another normotensive mouse strain (C57Bl/6) showed a similar duration of BP surge (1.7 ± 0.4 hr) compared to the Slagher BPH normotensive mice. These studies show that during the active period the hypertension is driven by a longer period of sustained activation of the BP surge which starts earlier and finishes later than in BPH normotensive counterparts. The mechanisms that regulate the timing of these changes may be key to the understanding of the cause of the hypertension in these mice.

SYMPATHTIC EXCITATION CAUSED BY PACAP-38 IN THE SPINAL CORD IS MEDIATED VIA THE PAC1 RECEPTOR

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A dual action drug containing both AT1 receptor antagonist and antioxidant properties would be a potential therapeutic for the disease atherosclerosis as it comprises of hypertension, inflammation and oxidative stress. The AT1 receptor antagonist mibefradil has been combined with several antioxidant pharmacophores (selenium, phenol and ebselen) with the aim that they will have dual actions. The AT1 receptor antagonist potency of these compounds has previously confirmed, with all of the antioxidant-substituted mibefradil analogues, except ebselen-mibefradil, showing antagonist properties in rat isolated right aorta. The present study compared these in vitro antioxidant properties in cell and tissue-based assays. The antioxidant properties of the compounds were examined in an AAPH (2,2′-azobis (2-amipropionic acid) hydrochloride)-induced haemolysis assay (mouse C57/BL6 isolated red blood cells) in the presence and absence of 1 mM glutathione (GSH). AAPH results in free radical-mediated haemolysis which is quantified by calculating the area under the absorbance over time curve. In the absence of GSH neither the antioxidant groups nor mibefradil analogues protected against haemolysis. However in the presence of GSH, ebselen, selenocystein, phenol and phenol-substituted mibefradil protected against lysis (N = 4 each; P < 0.05 vs. vehicle), whereas the other mibefradil analogues did not. For comparison, the antioxidant capacity of these compounds was also tested in a tissue-based preparation (mouse C57/BL6 isolated paired left atria) using doxorubicin (30 μM) as the free radical generator. Doxorubicin resulted in a percentage decrease in left atrial force of 65% over 90 min which can be concentration-dependently reversed by pre-incubating with the antioxidant quercetin (10, 30 and 100 μM). In this assay ebselen and phenol but not selenocystein (10 μM) each protected against doxorubicin-induced negative inotropy. The substituted mibefradil analogues did not protect against oxidative damage, however when pre-treating with mibefradil, phenol-mibefradil was able to protect against radical-mediated damage (N = 4 each; P < 0.05 vs. vehicle). The data from these experiments show that phenol-substituted mibefradil to longer analogues to have antioxidant properties that ebselen displays nor the antioxidant potency mibefradil possess. In contrast, the phenol-substituted mibefradil retains AT1 receptor antagonist potency but not antioxidant properties in the tissue-based assay as receptor binding interferes with the antioxidant moiety. Attempts to structurally modify this interaction may result in a dual action drug with therapeutic potential for the disease atherosclerosis.

CHARACTERISTICS OF EXAGGERATED CARDIOVASCULAR CIRCADIAN RHYTHM IN SCHLAGER HYPERTENSIVE MICE

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The morning surge in blood pressure (BP) is greater and rises faster in hypertensive patients at a time when there is a greater incidence of cardiovascular events. An underlying mechanism may be the exaggerated activation of the sympathetic nervous system. We have recently shown that the BPH (BP high) Schlagler mice are hypertensive due to an overactive sympathetic nervous system and have a greater day night differences in BP and heart rate compared to the BPH (BP normal). The aim of this study was to assess the characteristics of the surge in BP during the change from inactive to active periods in Schlagler BPH mice. Radio-telemetry devices were implanted in 11 normotensive and 10 hypertensive mice and after 10 days recording 24h hours of continuous recording, a circadian algorithm was fitted to the individual data sets to estimate day and night plateau as well as the rate of change in variables during dark cycle light. The diurnal range in BP and heart rate were 134% and 68% greater respectively in BPH compared to BPH normotensive mice (P < 0.001). However, the rate of rise in BP from baseline was significantly different between the two strains (2.7 ± 0.02 vs 8.4 ± 0.19 mmHg/h). Thus the duration of the surge in BP was markedly longer in the BPH hypertensive animals (3.2 ± 0.5 vs 1.8 ± 0.4 hr; P = 0.02) but the midpoint of the rise was similar. Comparison with another normotensive mouse strain (C57Bl/6) showed a similar duration of BP surge (1.7 ± 0.4 hr) compared to the Slagher BPH normotensive mice. These studies show that during the active period the hypertension is driven by a longer period of sustained activation of the BP surge which starts earlier and finishes later than in BPH normotensive counterparts. The mechanisms that regulate the timing of these changes may be key to the understanding of the cause of the hypertension in these mice.

CARDIAC AND ANTIOXIDANT EFFECTS OF NOVEL ANGIOTENSIN II RECEPTOR ANTAGONISTS IN VITRO

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Dynamic synchrontron imaging of diabetic rat coronary microcirculation in vivo

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Endothelial dysfunction in the diabetic coronary circulation plays an important role in the subsequent decline in heart function. Conventional angiography is not adequate to assess impairments in micro-vascular function. Using synchrontron imaging we are now able to detect small vessel calibres (≤40 μm, vs. 200 μm using a conventional X-ray device) and quantify regional differences in calibre under conditions of high heart rate (≤500 m/s). Experiments were conducted at the Japanese Synchrontron, SPring-8 using anaesthetised Sprague-Dawley rats 3 weeks after treatment with vehicle (n = 8) or streptozotocin (65 mg/kg iv. n = 11). Indocyanine green contrast medium (350 mg/ml) was injected into the coronary circulation. Using cine-radiograms and temporal subtraction we investigated endothelium-dependent and -independent vasodilatory responses in individual coronary vessels. Our results suggest that one of the coronary vasodilatory mechanisms is dysfunctional as both segmental vasconstriction and focal stenoses were seen after nitric oxide synthase (NOS) and cyclooxygenase (COX) blockade in streptozotocin rats (Fig. 1, black arrows), but not vehicle rats. Thus, in the early diabetic state streptozotocin treated rats showed localised coronary impairment. Synchrontron imaging provides a novel method to investigate coronary microvascular function in vivo,
in disease models. These findings further indicate that in the early diabetic state there is already localised coronary endothelial dysfunction.

**Figure 1:** Synchrotron angiogram depicting non-uniform acetylcholine induced vasodilation of coronary vasculature in diabetic rat after NOS and COX blockade.

### NITROXYL (HNO), A NOVEL REDOX SIBLING OF NITRIC OXIDE (NO\(^{-}\)), WITH VASOPROTECTIVE ACTIONS

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The therapeutic utility of the NO\(^{-}\)/cGMP pathway has long been recognised with nitrovasodilators such as glycyltrinitrate (GTN) used for the treatment of cardiovascular disorders such as angina, hypertension and heart failure for >100 years. The clinical efficacy of traditional NO\(^{-}\) donors is limited, however, by susceptibility to non-enzymatic degradation and therefore requires the development of NO\(^{-}\) derivatives with increased bioavailability and reduced tissue levels of degradation products. HNO donors such as Arginine and IPA/NO target predominantly the soluble guanylyl cyclase (sGC)/cGMP signaling pathway to mediate vasodilation. Moreover, in contrast to GTN, HNO donors do not develop vasoreactivity and may provide innovative pharmacotherapy for the treatment of vascular disease.

**ARGINASE II DEFICIENT MICE DO NOT DEVELOP NITRATE TOLERANCE: ENHANCED BASAL REACTIVE OXYGEN SPECIES**

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Arginase competes with endothelial nitric oxide synthase (eNOS) for L-arginine, therefore regulating nitric oxide (NO) production in the vasculature. Recently we demonstrated the reduction of nitrate tolerance in the presence of non-isofrom specific arginase inhibitors. Here we hypothesise that arginase II inhibition can reduce nitrate tolerance via preservation of intracellular L-arginine and the reduction of reactive oxygen species (ROS). Aortae from wild type (WT) and ArgIIKO mice and arginase II knockout (ArgIIKO) mice were mounted in myographs and cumulative concentration-response curves (CRCs) to NO donors, glyceryl trinitrate (GTN) or sodium nitropusside (SNP) conducted. A second CRC, 30 min later showed WT mice developed tolerance to GTN and SNP, with a 32-fold and 5-fold shift to the right in the CRCs, respectively (n=9, P<0.001). In contrast, tolerance to GTN in n=6, P<0.05) and SNP in n=8 (P<0.05) was not evident in ArgIIKO mice. In vivo GTN tolerance was induced by injecting GTN (20 mg/kg s.c.) 8 hourly for 3 days. A vehicle (5% glucose) control was used. Tolerance was evident in GTN treated WT aorta with a 7-fold shift in the CRC (Vehicle: E\(_{\text{max}}\)=8.8±2.2; vs GTN: E\(_{\text{max}}\)=51±6.3, P<0.05), while in ArgIIKO aorta it was not (P>0.05). GTN-tolerant vessel volumes have increased ROS production (P<0.05), assessed by lucigenin-enhanced chemiluminescence, when compared to non-tolerant vessels, while there was no difference in ArgIIKO tolerant vs non-tolerant vessels (P>0.05). Importantly, basal ROS production is increased in aortae of ArgIIKO mice compared to WT mice (P<0.01). Phosphorylation of vasodilator-stimulated phosphoprotein (VASP), determined by western blot analysis, in aortae of non-tolerant ArgIIKO mice, was reduced (P<0.05) and comparable to WT tolerant vessels. In conclusion, nitrate tolerance is absent in ArgIIKO mice but this may be due to the already enhanced basal ROS production.

### AREA PRODUCT

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By definition, Critical Closing Pressure (CCP) and Resistance Area Product (RAP) are evaluated from pressure and flow waveforms that are measured at the same location. However, due to non-invasive measurement error and estimation error, radial arterial pressure waveform (ABP) and with cerebral flow velocity (FV) waveforms, there is an inherent time lag between recorded signals due to transmission times over different path lengths. This study aims to quantify the effect of the time lag between ABP and FV with cerebral flow velocity (CFC) on the calculation of CCP and RAP. Calculations were performed on sequential cardiac cycles (n=67) of simultaneous recordings of radial pressure waveforms and right middle cerebral artery (MCA) flow velocity waveforms using application tonometry and transcranial Doppler respectively in a single healthy female subject (32 y.o). CCP and RAP were computed for each cardiac cycle using the first systolic component of ABP and CFC waveform, and backwards by 10, 20, 30, 40 and 100 msec to simulate the time lags and CCP and RAP calculated as a function of time lags. Mean and SD of CCP and RAP values for each time
lag are shown in the boxplot figure below. CCP and RAP values from –30 to 20 msec shift appear to be not statistically different to the zero-shifted datasets.

**ASSESSING THE MAGNITUDE OF THE STRUCTURAL TPR-AMPLIFIER IN PAGE HYPERTENSION IN CONSCIOUS RABBITS**

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There has been considerable controversy whether structural changes in the resistance vessels in hypertension enhance vascular resistance (R) responses to constrictor and dilator stimuli. Our group and many investigators have provided evidence from *in vivo*, *in vitro* and model studies. However, others could not confirm experiments pertaining to the total peripheral resistance (TPR) amplifier. To help resolve the controversy we reanalysed an earlier in vivo study 1 in 15 conscious rabbits; both kidneys had been wrapped in cellophane and an aortic Doppler flowmeter for measuring cardiac output (CO) and a left atrial catheter for infusing vasoactive drugs were implanted 5 and 3 weeks before starting experiments. The rabbits were studied on 3 days: 1) with all effects intact; 2) during ganglionic blockade (GB) with mecamylamine; 3) during neuronal blockade (NBH), which eliminated activity of the ANS and the pressor hormones Ang II and AVP. As agonists we infused AngII, methoxamine, acetyl choline and adenosine, to derive extended scaled dose (SCD) – total peripheral conductance (TPC) and – TPR response curves. Earlier only the TPC curves were examined, and the slope ratios (H/N) between hypertensive and normotensive animals determined by linear regression. As a result we missed two major non-linearities that were more obvious in the TPR curves. On this occasion both curves were examined by polynomial regression, which accounted for 85–95% of their variance. The non-linearities were due to: 1) functional rarefaction associated with reduction of CO at high doses of constrictors; 2) hypotension caused by high doses of dilators, which limited autoregulatory capacity. Both confound assessment of the structural amplifier. Between these non-linearities there remained a substantial dose-range for assessing dilators, which limited autoregulatory capacity. Both confound assessment of the structural amplifier.

**THE MECHANISM OF ANTI-APOPTOTIC ACTION OF MINERALOCORTICOID RECEPTOR BLOCKADE IN EXPERIMENTAL MYOCARDIAL INFARCTION**

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Heart disease is the leading cause of death in Australia. Although thrombolytic therapy and percutaneous coronary interventions reduce mortality from acute myocardial infarction, additional therapeutic strategies are needed. Clinical trials (RALES & EPHESUS) have shown mineralocorticoid receptor (MR) antagonists added to standard of care substantially increase survival and decrease hospitalization in heart failure. We have previously shown cardiac damage during experimental myocardial infarction is aggravated by the MR agonists aldosterone or cortisol and reversed by 1 μM spironolactone. In addition, spironolactone alone reduced myocardial damage resulting from ischemia-reperfusion (IR) injury. Since reperfusion injury has been correlated with increased apoptosis in the area at risk, we examined whether regurger spironolactone apoptosis mediates the cardioprotective effect of MR blockade. Sprague–Dawley (SD) rats were anesthetized, the hearts isolated and subjected to regional ischemia followed by reperfusion. MR antagonists, spironolactone (SP, 1, 3, 10 and 1000 nM) or eplerenone (EPL, 100 and 1000 nM) were added to perfusates prior to inducing ischemia and maintained throughout the reperfusion period. At the completion of reperfusion, infarct area and apoptotic index were measured. Apoptotic markers, active caspase-3, acinus, and anti-apoptotic protein, ARC were detected by immunostaining. Spironolactone superfluous alone significantly reduced infarct size (35 ± 2%, N = 8 vs 41 ± 1%, N = 7, P < 0.05) and the number of TUNEL-positive cardiomyocytes (8.4 ± 0.8%, N = 7 vs 7.7 ± 0.6%, N = 8, P < 0.05) via inverse agonist activity at mineralocorticoid receptors, an effect near-maximal at relatively low dose (10 μM). Although eplerenone (100 nM and 1 μM) reduced infarct size (mean values of 37 ± 2%, N = 9 and 36 ± 2%, N = 8, respectively) this was not significant (P > 0.10 & 0.09). Consistent with the reduction in TUNEL staining, spironolactone reversed both active caspase-3 and acinus processing during IR. Spironolactone also regulated anti-apoptotic protein ARC (apoptosis repressor with a caspase recruitment domain) activity. ARC expression was significantly reduced during IR (1.0 ± 0.06 vs. 0.9, N = 7 vs 2.2 ± 0.3, N = 7, P < 0.05) and restored

**EFFECTS OF G Hernandez INFUSION ON SYMPATHETIC NERVOUS SYSTEM ACTIVITY IN HUMANS**

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Ghrelin is a recently discovered GH-releasing peptide secreted by the stomach with effects on appetite and cardiovascular regulation. Animal studies suggest that ghrelin acts centrally to decrease food intake and increase sympathetic nervous system (SNS) and may contribute to the stress induced cardiovascular responses. To investigate the effects of ghrelin in humans, we gave 9 lean healthy men (age 21 ±0.3 years) an intravenous infusion of human ghrelin (5pmol/kg/min for 1 hour) and saline in a randomized fashion. Ghrelin elicited a small decrease in systolic and diastolic blood pressure (~12 mmHg) and HR (12–17 bpm) which are not significantly different compared with saline infusion (~15 mmHg and ~11% in MSNA). These results indicate that in healthy human, ghrelin-induced decrease in blood pressure is accompanied by a marked increase, rather than a decrease, in SNS activity. We hypothesize that ghrelin activates the SNS through baroreceptor unloading as a result of a peripheral mechanism. To investigate this, we assessed heart rate variability (HRV) before and after 60 min of ghrelin and saline infusion and during different mental arithmetic tasks (3 bursts per min, 2 to 10 sec) and mental arithmetic tasks (3 bursts per min, 2 to 10 sec). HRV increased with ghrelin infusion but not saline infusion. We concluded that ghrelin activated the SNS through baroreceptor unloading as a result of a peripheral mechanism.
by 10 nM spironolactone (1.9 ± 10^−6 M; N = 8). Conclusion: Spironolactone acts merely by excluding corticosteroids from mineralocorticoid receptors, but as a protective inverse agonist at low concentration and by activation of anti-apolipoprotein protein(s).

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INFLUENCE OF ALTERING DIETARY n-6:n-3 POLYUNSATURATED FATTY ACID RATIO ON MARKERS OF VASCULAR HEALTH IN PATIENTS TREATED WITH STATINS: A RANDOMISED CROSSOVER TRIAL

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Increasing the dietary intake of n-3 polyunsaturated fatty acids (PUFAs) may decrease the risk of coronary heart disease. However, n-6 PUFAs appear to compete with n-3 PUFAs for common mechanistic determinants, in part related to the dogmatic focus on renal sodium homeostasis and blood pressure control of sodium homeostasis and BP, i.e. aldosterone-mineralocorticoid receptors (MR) – Na+/K+ -ATPase have now been demonstrated to act in the central nervous system. This pathway is being regulated independently of the peripheral/renal pathway and contributes to regulation of cerebral spinal fluid (Na+) by the choroid plexus, of brain tissue [Na+] by the ependyma and to neuronal responses to e.g. Na+ or Ang II. Increases in CSF [Na+] by central infusion of Na+ rich aCSF or by high salt intake in Dahl S SHR cause sympathetic-excitation and hypertension. These studies indicate a need to better understand how to intervene to decrease chronic levels of salt intake, irrespective of the presence of a “salt-sensitive kidney”. We propose that in salt-sensitive hypertension an increase in CSF [Na+] causes a local increase in aldosterone biosynthesis which activates an aldosterone dependent neuromodulatory pathway which enhances activity of angiotensinergic sympatho-excitation pathways leading to hypertension. Looking beyond the kidney is providing new insights into mechanisms contributing to salt-sensitive hypertension, which will help to dissect the genetic factors involved and to discover novel strategies to prevent and treat salt-sensitive hypertension.

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LARGE ARTERY STIFFNESS INCREASES WITH LOCAL NEUROGENIC BLOCKADE IN RATS

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Whilst the functional role of neurogenic control of peripheral blood vessels is well established, the presence of neurogenic terminals in the large arteries has not been linked with changes in large artery stiffness. The aim of this study was to evaluate any change in rat aortic stiffness, through measurement of pulse wave velocity (PWV), as a result of local neurogenic blockade. Methods. Male Sprague-Dawley rats (n=7, aged 12-14weeks) were anaesthetised and two 1.3F, high fidelity pressure sensors introduced into the proximal and distal ends of the abdominal aorta for measurement of PWV. Increasing doses (1µg/ml, 10µg/ml & 100µg/ml of an alpha-adrenergic antagonist (phentolamine) were used to locally chemically denervate the abdominal aorta via bathing through a ventrally exposed cavity. Mean arterial pressure was raised and lowered using intravenously injected phenylephrine and sodium nitroprusside respectively. PWV was measured across the full physiological arterial pressure range, and during the different levels of local neurogenic blockade. Results. The increasing concentrations of phentolamine caused aortic PWV to increase concordantly. Neurogenic blockade caused a significant change in PWV at arterial pressures above 110mmHg, with PWV increasing from 5% (1µg/ml phentolamine) to 10% (10µg/ml phentolamine) compared to control values (P<0.05). The response was more pronounced at aortic pressures above 130 mmHg, with 10µg/ml phentolamine resulting in a 12.5% increase in PWV compared to control (P<0.01). Conclusions. These results show that local neurogenic blockade in large arteries can significantly increase local arterial stiffness. This demonstrates that neurogenic input into large arteries results in functional physiological changes that could impact on blood pressure regulation.

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SINGLE DOSE WHOLE-BODY IRRADIATION CAUSES ACUTE AND REVERSIBLE INCREASES IN LARGE ARTERY STIFFNESS IN RATS

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Exposure to high energy radiation causes cardiovascular injury. It has been demonstrated that whole-body gamma irradiation impairs the endothelial-dependent vasoconstrictor function. Such impairment may cause increased arterial stiffness, as can be shown by increased pulse wave velocity (PWV). The current study aims to determine the effects of whole-body high-dose high-energy X-ray on aortic PWV. Male Sprague-Dawley rats (14 weeks old) were exposed to a single high-dose of high-energy X-ray (source: LINAC, 6 MV) at either 0 (control) or 5 Gy (irradiated). Between 0 to 24 hours, 24 to 48 hours and 3 weeks following irradiation, rats were anaesthetized (1.3 g/kg, urethane) and beat-to-beat PWV (m/s) and mean arterial blood pressure (MAP) measured invasively using two 1.3F high fidelity pressure sensors (Scolaire Inc, Canada) positioned in the descending thoracic aorta via the right femoral and left carotid arteries. Arterial pressure was increased and decreased over the range of 60-150 mmHg with intravenous infusion of phenylephrine and sodium nitroprusside respectively. Across the blood pressure range, mean aortic PWV in the irradiated rats measured within 24 hours post-irradiation was 5.08±0.15 m/s, a 14% increase compared to the control group (4.4±0.11 m/s; n=12; P<0.05). No significant difference was observed between the PWV in the irradiated group measured in the 24-48 hour period post-irradiation and the control group (4.14±0.10 m/s; n=4; P=0.44). Similarly, no difference was found in the arterial stiffness of control and irradiated rats 3 weeks after radiation (control –4.37±0.21 m/s irradiated –4.53±0.13 m/s; n=12; P=0.41). Whole-body exposure to a single dose of high-energy X-ray increased aortic stiffness in the period of 0 to 24 hours after the radiation treatment. These changes were reversed in the subsequent 24 hour period, and remained for 3 weeks following irradiation. These findings suggest an acute mechanism behind endothelial impairment associated with whole-body, high-dose irradiation. Further research is required to ascertain the exact nature of that mechanism.

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SALT-SENSITIVE HYPERTENSION: TIME TO CHANGE THE PARADIGM

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High dietary salt intake is a major “lifestyle factor” contributing to the progressive increase in BP with ageing in most Western Societies. Despite extensive research, the genetic and environmental determinants are still poorly defined, in part related to the dogmatic focus on renal mechanisms. Na+–transport regulating mechanisms classically considered to reflect renal control of sodium homeostasis and BP, i.e. aldosterone-mineralocorticoid receptors (MR) –
IDENTIFICATION OF RENIN-ANGIOTENSIN SYSTEM (RAS) IN HUMAN FETAL MEMBRANES, DECIDUA AND PLACENTA AND THE EFFECTS OF GENDER AND LABOUR

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Human intrauterine tissues and amniotic fluid contain prorenin. Amniotic (pro)renin can generate Ang I from angiotensinogen (AGT) or stimulate cell signalling pathways directly when bound to the (pro)renin receptor (P)RR. To measure the expression of RAS components, including prorenin, (P)RR, AGT, ACE1, ACE2, AT1R, AT2R, MAS1, angiotenin, chorion, decidua, and placenta were collected before and after labour from 24 women. RNA was extracted and real-time PCR used SYBR Green for detection, with abundance calculated relative to β-actin mRNA using the 2-ΔΔCt method. Immunohistochemistry was performed using specific antibodies to localize RAS proteins in membrane and placental sections. Prorenin mRNA abundance was highest in decidua (P<0.001), being highest in decidua collected directly after labour from pregnancies that carried a female fetus (P=0.06). After labour, there was a significant decrease in decidua prorenin mRNA in pregnancies carrying a female fetus (P<0.035). (P)RR mRNA was highest in placenta (P<0.001), AT1R mRNA was highest in decidua (P<0.003). ACE1 mRNA was lowest in fetal membranes (P<0.001) and higher in decidua than in placenta (P<0.003). ACE2 mRNA was lowest in fetal membranes (P<0.001) and highest in decidua (P<0.002). AT1R mRNA was highest in placenta (P<0.001). AT2R and MAS1 receptor mRNAs were not detected. It is concluded that in amniotic, mRNA expression of RAS components is low, but (P)RR is abundant and may bind protein from amniotic fluid or decidua. In decidua, RAS components are abundant (except AT1R). In placenta, since ACE1 components is low, but (P)RR is abundant and may bind prorenin from amniotic fluid or decidua. In placenta, RAS components are abundant (except AT1R). In placenta, since ACE1 protein is localized to fetal capillary endothelial cells and AT1R mRNA and protein are present, syncytiotrophoblast may contain 2 independent RAS pathways, one in fetal vessels and one in syncytiotrophoblast. The placenta may be affected by maternal Ang II. Therefore fetal membranes may not generate Ang II, and RAS actions may be mediated directly through the (P)RR. Decidua and placenta may be able to generate Ang II and Ang 1–7.

SIGNALLING FOR ANGIOGENESIS IN BRAIN REPAIR FOLLOWING ISCHAEMIC STROKE AND REPERFUSION

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NADPH oxidase-derived reactive oxygen species (ROS) contribute to the progression of acute brain injury following ischaemic stroke. Despite this, ROS may also regulate endogenous regenerative mechanisms that occur in the brain after stroke. Angiogenesis in the damaged brain is crucial to support survival and newly developing neurons. We are searching for molecular targets to enhance these endogenous repair mechanisms that might aid the timely delivery of targeted stem cell therapy. We used rodent models of transient stroke and investigated histological and functional outcomes to determine the timecourse of angiogenesis in the brain after reperfusion ischaemic stroke. We have also identified angiogenic and oxidative signaling factors involved in this response in rats. Functional deficits in behaviour were detected between 1 and 7 days post-stroke (P<0.01). Blood vessel numbers decreased within the cortical infarct core 6 h after stroke (30±10% vs 0.05), but by 14 and 28 days numbers increased markedly in the cortical infarct core (64±4% and 76±6 %) and moderately increased in the cortical border zone (19±5% and 23±7 %) respectively, compared to contralateral brain regions (P<0.05). Double immunofluorescence labelling revealed that the marked increase in blood vessels in the infarct core at 14 and 28 days was associated with DHE-detectable superoxide generation and only occurred in brain regions that had lost all neurons. Real-time PCR detection of NADPH oxidase (Nox) subunits revealed a marked increase in Nox2 mRNA expression in the ipsilateral cortex up to 7 days post-stroke (up to 40 fold P<0.001) which returned to normal by 14 days. Nox4 mRNA was significantly increased later at 14 days. Angiogenic factor VEGF mRNA was also increased in the cortical infarct cortex between 7 and 14 days post-stroke (up to 20 fold P<0.05) but returned to normal levels by 28 days. Transplantation of stem cells into the brain post-stroke is currently thought to be an exciting treatment option. Optimisation of the damaged brain environment after stroke is crucial for the survival of transplanted cells. NADPH oxidases may be involved in the regulation of angiogenesis in the brain in the weeks following ischaemic stroke and reperfusion and therefore present a target for enhancement of brain repair.

COMPARISON OF THE EFFICIENCY OF THE INFLUENCE OF THE ANTI-HYPERTENSION THERAPY ON THE SPEED OF GLOMERULAR FILTRATION IN PATIENTS WITH METABOLIC SYNDROME

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The result of the modern style of life (lack of physical activity, surplus use of high caloric food etc.) is the increase of patients with type II diabetes, obesity, heart diseases and disruption of lipid exchange. In different countries the rate of people who suffer from metabolic syndrome is 20—65%. In this group of people the level of death is twice higher and level of cardiovascular symptoms three times higher than in patients without metabolic syndrome. In patients with arterial hypertension, function of the renal system decreases by 1% per year, and arterial hypertension together with diabetes – by 13%. The aim of the study was to compare the influence of anti-hypertension therapy on the speed of glomerular filtration. There were 39 patients with metabolic syndrome 33–83 years old. Patients underwent the following examination that measured: the body index, circumference of the waist, circumference of the thigh, sagittal size of the stomach, percentage of body fat, arterial heart rate, blood sugar, triglycerides and general cholesterol in blood, echocardiography in B and M regimes, creatinine level and creatinine clearance, and the glomerular filtration rate (GFR). Patients were divided into two groups, and they were all treated with standard therapy for metabolic syndrome, including hypoglycemic medication and statins. Groups received different antihypertensive medication. The first group of patients received an angiotensin converting enzyme inhibitor (ACE). At the beginning of the investigation there was no significant difference between the measured variables. During the experiment, the GFR significantly increased in both groups. In the ARB group, GFR increased from 77.92±13.63 ml/min to 91.46±10.55 ml/min (n=20; P=0.03). In the ACE group, GFR increased from 75.24±12.97 ml/min to 86.37±11.15 ml/min (n=19; P=0.04). Comparison of the ARB and ACE groups after treatment indicated that GFR was higher in the ARB group than in the ACE group (n=39; P=0.03). Thus, antihypertensive therapy increases GFR in the setting of metabolic disorder. Interestingly, the results suggest that angiotensin receptor blocker blockade is more effective for increasing GFR than angiotensin converting enzyme inhibition.

ENDOTHELIAL PROGENITOR CELLS AND THE CAROTID INTIMA-MEDIA THICKNESS IN SEVERE OBESITY

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Endothelial progenitor cells (EPC) are primitive cells that are important in endothelial repair and regeneration in blood vessel walls following injury. Their impairment is associated with cardiovascular disease (CVD) and risk factors. It is well regarded in non-obese populations that EPC levels serve as a cellular biomarker of CVD. However, EPC level and role in severe obesity are yet to be established. To explore whether EPC could function as a cellular biomarker of CVD in the severe obese, a correlation study was carried out. In this study we assessed EPC number.
and function and carotid intima-media thickness (IMT), an established non-invasive marker of atherosclerosis, in 63 severe obese subjects (46.3 ± 1.1 yrs, BMI 45.2 ± 0.7 kg/m²), and 26 age and gender-matched controls (48.1 ± 2.3 yrs, BMI 25.5 ± 0.5 kg/m²). Circulating EPC level was determined by FACS counting of percentage of AC133⁺/KDR⁺ cells in 10⁵ Ficoll-density isolated peripheral mononuclear cells, and EPC-colony forming unit (EPC-CFU) was assessed using a standard EPC-CFU assay. EPC number and EPC-CFU were increased 1.7-fold (0.089 ± 0.011% versus 0.51 ± 0.011%, P = 0.04) and 3.4-fold (11.1 ± 1.9 colonies/well vs 3.3 ± 1.1 colonies/well, P = 0.03) respectively, in the severely obese compared to controls. IMT was greater in the severely obese subjects (0.658 ± 0.016 mm vs 0.570 ± 0.016 mm, P = 0.001) compared to controls. Correlation analysis revealed that EPC number was positively associated with IMT in the controls (P = 0.02) but not in the obese while EPC-CFU did not show any correlation. These results indicate that in severe obesity there is no impairment of EPC function, suggesting that EPC are not an adequate cellular biomarker of CVD in this population. These findings illustrate the complexity of the pathophysiology in the severe obese. Meanwhile the pituitary-adrenal axis following prenatal ethanol exposure, which is known to be affected by ethanol exposure during pregnancy, whilst having minor effects on basal blood pressure, was greater in the severely obese subjects (0.658 ± 0.016 mm vs 0.570 ± 0.016 mm, P = 0.001) compared to controls. Restraint stress increased MAP by 30 –35 mmHg in control offspring (n = 33.3 ± 0.6 g/kg; 1.2 ± 0.01 mg/kg) and a single dose of FR139317 (1.5 mg/kg), an ETAR antagonist, was administered intravenously to each fetus and the MAP and HR responses were recorded. In a second study, three different doses of endothelin-1 (ET-1) (0.4 µg/kg, 0.8 µg/kg and 1.2 µg/kg) and a single dose of FR139317 (1.5 mg/kg), an ETAR antagonist, were administered intravenously to each fetus and the MAP and HR responses were recorded. In a separate cohort of 9 Control and 9 PR fetuses, coronary arterioles were collected at 137–142 d gestation, snap frozen and then the RNA was extracted and real-time PCR as used to measure NGF, ETAR and ETBR gene expression. These data suggest that ET does not play a different role in maintaining blood pressure in the PR fetus and suggest a role for NGF in regulating coronary blood flow in the PR fetus.

MODERATE PRENATAL ETHANOL EXPOSURE IMPAIRS THE PRESSOR RESPONSIVENESS TO STRESS

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Chronic prenatal exposure to high levels of ethanol can result in impaired fetal development. This may manifest as altered growth, impaired cardiac development and altered sensitivity to stress. Less is known about the impact of more modest ethanol exposure on fetal development. We examined the effects of a moderate prenatal ethanol treatment (PET, 15% caloric from ethanol), administered throughout pregnancy in the rat, on fetal and postnatal growth. At 12 months of age, basal blood pressure, heart rate and activity were measured using radiotelemetry and pressor responses to restraint stress was examined. Both males and females were examined. On day 20 of pregnancy, female PET foetuses were growth restricted (n = 8–9 litters/group, P < 0.05). However, there was no effect of PET on body weight at birth or its slope of change over gestation. Females were grouped by birth weight into light (< 55% of control) and heavy (≥ 55% of control) groups. In male and female foetuses, PET males had lower heart rates than control males at all times. PET females had lower heart rates only at < 30 days of age. Restraint stress increased MAP by 30–35% in control offspring but there was no effect of PET. Locomotor activity was decreased in female PET offspring was significantly lower than control offspring (n = 3.3 ± 0.1% vs 5.1 ± 0.1%, P < 0.011% respectively, in the severely obese compared to controls. IMT was greater in the severely obese subjects (0.658 ± 0.016 mm vs 0.570 ± 0.016 mm, P = 0.001) compared to controls. Restraint stress increased MAP by 30–35 mmHg in control offspring (n = 33.3 ± 0.6 g/kg; 1.2 ± 0.01 mg/kg) and a single dose of FR139317 (1.5 mg/kg), an ETAR antagonist, was administered intravenously to each fetus and the MAP and HR responses were recorded. In a second study, three different doses of endothelin-1 (ET-1) (0.4 µg/kg, 0.8 µg/kg and 1.2 µg/kg) and a single dose of FR139317 (1.5 mg/kg), an ETAR antagonist, were administered intravenously to each fetus and the MAP and HR responses were recorded. In a separate cohort of 9 Control and 9 PR fetuses, coronary arterioles were collected at 137–142 d gestation, snap frozen and then the RNA was extracted and real-time PCR as used to measure NGF, ETAR and ETBR gene expression. These data suggest that ET does not play a different role in maintaining blood pressure in the PR fetus and suggest a role for NGF in regulating coronary blood flow in the PR fetus.

HEART RATE, BUT NOT BLOOD PRESSURE, RESPONSE TO ENDOTHELIN-1 RECEPTOR BLOCKADE IS GREATER IN GROWTH RESTRICTED FETUSES

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Small size at birth is associated with an increased risk of perinatal and postnatal cardiovascular morbidity and mortality. Growth restricted fetuses have an increased dependence on the sympathetic nervous system in the maintenance of mean arterial pressure (MAP). Endothelin-1 (ET) mediates cardiac sympathetic innervation by regulating the gene expression of nerve growth factor (NGF). The impact of PR on development of the ET system and the contribution of ET-1 to MAP regulation are not known. The aim of this study was to compare the effect of ET-1 blockade on MAP and HR and the mRNA expression of NGF, ETAR receptor and ETBR receptor in control and growth restriction (PR) fetuses. Prenatal restriction (PR) leading to fetal growth restriction was induced by removing the majority of the endometrial caruncles from Merino ewes prior to mating. Vascular catheters were implanted at 120–130d gestation in Control (n = 6) and PR (n = 5) fetuses. Three different doses of endothelin-1 (ET-1) (0.4 µg/kg, 0.8 µg/kg and 1.2 µg/kg) and a single dose of FR139317 (1.5 mg/kg), an ETAR antagonist, were administered intravenously to each fetus and the MAP and HR responses were recorded. In a separate cohort of 9 Control and 9 PR fetuses, coronary arterioles were collected at 137–142 d gestation, snap frozen and then the RNA was extracted and real-time PCR used to measure NGF, ETAR and ETBR gene expression. ET-1 increased MAP at a lower dose in Control (0.8 µg/kg) compared to PR (1.2 µg/kg) fetuses but there was no difference in the magnitude of the rise. Fetal MAP and HR responses during FR139317 infusion were not different between control and PR fetuses. NGF, but not ETA or ETBR receptor, gene expression in the heart was higher in PR compared to control fetuses. There was no relationship between NGF and ETAR or ETBR gene expression. These data suggest that ET does not play a different role in maintaining blood pressure in the PR fetus and suggest a role for NGF in regulating coronary blood flow in the PR fetus.
DIFFERENCES IN PERSISTENCE WHEN CALCIUM CHANNEL BLOCKERS ARE COMBINED WITH ANGIOTENSIN CONVERTING ENZYME (ACE) INHIBITORS

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The NHF Guide to management of hypertension (2008) states that “based on the best available evidence, the most effective combination is an Angiotensin Converting Enzyme [ACE] inhibitor or an Angiotensin II Receptor Antagonist [A2RA] plus a calcium channel blocker [CCB]”. PBS claims data provided by Medicare Australia has been used to assess persistence to ACE/CCB (dihydropyridine CCB) combinations. This analysis is based on all scripts supplied to a one in ten sample of the Australian patient population drawn from de-identified Medicare Australia claims data. Only Concessional patients were included because many AHT products fall under the General copayment. Patients may be misleading since the intervals between repeats suggest that most patients are compliant if they collect their prescriptions. Interventions should be targeted towards ensuring that patients collect their scripts, rather than improving adherence.

Figure 1. Persistence to DHP/A2RA combinations.
were evaluated using telemetric BP recordings. The gain of the transfer function between systolic BP and pulse intervals used to estimate the spontaneous BRS (ms/mmHg) was calculated in the low frequency (0.15–0.60 Hz) band. Transgenic AT1a MUT exhibited higher BP and heart rate (HR) levels compared to controls (systolic BP AT1a MUT 134.6±5.9 mmHg; AT1aWT 110.5±5.9; P<0.05; HR AT1a MUT 531±15 at AT1a WT 455.5±5 beats/min; P<0.001). The spontaneous BRS was decreased in transgenic compared to wildtype (1.23±0.17 at AT1a MUT vs 1.91±0.18 ms/mmHg; P<0.05). Motor activity did not differ between groups. These variables exhibited circadian changes and the differences between the strains were maintained throughout the cycle. The highest values for BP, HR and locomotor activity were observed at night. The lowest BP levels in transgenic were the lowest of the entire day (6:00) with systolic at 104.2±7.0 mmHg and diastolic at 54.4±3.1 mmHg; P<0.001. BP and HR were elevated i.e. at night, when the animals were active. The BP power in the autonomic band, which in mice is an index of sympathovasomotor function, is increased significantly in mutant mice. In addition to the expected peripheral renal and vascular effects following constitutive activation of AT1a receptors, these studies provide new evidence for a sustained control of central sympathovasomotor pathways and also inhibition of the cardiac baroreflex which may contribute to the hypertension.

**SHOULD TREATMENT FOR GLUCOCORTICOID-SUPPRESSIBLE HYPERALDOSTERONISM (GSH) BE COMMENCED LONG BEFORE HYPERTENSION DEVELOPS, AND, IF SO, WHICH?**

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Since GSH can be diagnosed at birth in known families using cord blood DNA, an important question is when to commence treatment in order to prevent unwanted cardiovascular changes due to aldosterone excess. Since our studies in eight young normotensive affected siblings reported five years ago revealed echocardiographic abnormalities compared with age and sex matched normal controls, our objective was to examine the consistency and possible progression of already described disturbances in cardiac structure and function in normotensive individuals with GSH. Six of eight subjects with genetically proven GSH who had previously been shown to have structural and functional changes on echocardiography (compared with 24 age- and sex-matched normotensive controls) were restudied after 30.7±14.2 months of follow-up with measurement of office blood pressure (BP) and echocardiographic characteristics, including left ventricular (LV) wall thicknesses, parameters of LV diastolic filling and systolic function. Compared with the initial, previously reported evaluation, mean systolic BP remained similar (130.6±12.7 to 124.8±15.8 mmHg; P=0.5707) and diastolic BP increased (72.0±9.6 vs 80.3±14.5 mm Hg; P=0.0178). LV posterior wall (0.83±0.09 vs 1.01±0.13 cm; P=0.0185), LV mass (123.9±19.5 vs 190±3.57 g; P=0.0153), LV mass index (72.4±5.7 vs 103.6±20.8 g/m2; P=0.0102) and mitral inflow deceleration time (75.5±30.7 vs 203.7±35.6 ms; P=0.0921) increased after follow-up. There were no significant differences in LV diameters and volumes, interventricular septum, ejection fraction, CIVB, E/A wave ratio and E/E’ ratio. In GSH, aldosterone excess is associated with increased LV wall thicknesses, LV mass and reduced diastolic function, suggesting that specific treatment (either partial ACTH suppression or aldosterone blockade) should be commenced early and perhaps even long before hypertension develops. Given possible growth-retarding effects of glucocorticoids in children, and lack of perfect receptor specificity of spironolactone, the time has come to commence partial ACTH suppression or aldosterone blockade (of sufficient magnitude) should be commenced early and perhaps even long before hypertension develops. Given possible growth-retarding effects of glucocorticoids in children, and lack of perfect receptor specificity of spironolactone, the time has come to commence partial ACTH suppression or aldosterone blockade (of sufficient magnitude).

**UNILATERAL ADRENALECTOMY IMPROVES URINARY PROTEIN EXCRETION BUT DOES NOT ABOLISH ITS RELATIONSHIP TO SODIUM EXCRETION IN PATIENTS WITH ALDOSTERONE-PRODUCING ADENOMA**

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Experimental and human data suggest that the cardiorenal effects of aldosterone excess are dependent upon concomitant dietary salt intake. Increased urinary protein (Uprot) is an early sign of nephropathy independently associated with cardiovascular risk. In a previous study we demonstrated that there is a positive association between Uprot and urinary sodium (UNa) in patients with hyperaldosteronism, but not in patients with normal aldosterone levels. The objective of the current study was to determine if Uprot is related to UNa in patients with hyperaldosteronism, but not in patients with normal aldosterone levels. The activation of the sympathetic nervous system (SNS) through the central actions of the adipokine leptin has been suggested as a major mechanism by which obesity contributes to the development of hypertension. However, direct evidence for elevated sympathetic activity has been limited to muscle. The present study examined the renal sympathetic nerve activity (RSNA) and cardiovascular effects of a high fat diet (HFD) as well as the changes in the sensitivity to intravenous (i.v.) lepotin. New Zealand White rabbits fed a 13.5% FHD for 4 weeks showed modest weight gain but a 2–3 fold greater accumulation of visceral fat compared with control rabbits. Mean arterial pressure (MAP) and heart rate increased by +8% and +26% respectively and RSNA was +48% higher (P<0.05) as was plasma nonepinephrine concentration (+35%, P<0.05) following three weeks of HFD. Icv lepotin administration (5–100 μg) increased MAP similarly in both groups but RSNA increased more in HFD fed rabbits. By contrast, lepotin produced similar reductions in RSNA to control rabbits in regions important for appetite and sympathetic actions of lepotin (accurate ±54%, paraventricular –69% and dorsomedial hypothalamic –65%). These results suggest that visceral fat accumulation through consumption of a HFD leads to marked sympathetic activation, which is related to increased responsiveness to central sympathoexcitatory effects of lepotin. The paradoxical reduction in hypothalamic neuronal activation by lepotin suggests a marked ‘selective lepotin resistance’ in these animals.
term regardless of diet post-weaning. This study highlights how maternal nutrition can detrimentally impact on cardiovascular risk factors in the next generation.

074 IS EXERCISE BENEFICIAL FOR BLOOD PRESSURE AND METABOLIC RISK IN OFFSPRING FROM OBESE MOTHERS?

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Maternal obesity increases offspring cardiovascular risk. Physical exercise ameliorates diet induced fat gain, dyslipidemia and hypertension, and improves insulin resistance. We hypothesized that voluntary exercise would ameliorate the adverse effects of maternal obesity on central appetite regulators, lipid homeostasis and blood pressure in offspring. Sprague-Dawley females were fed a control diet (C) or high-fat diet (HFD). HFD then mated with males. At weaning, female offspring from C dams were fed chow (CC) and offspring from H dams were fed chow (HC) or HFD (HH). Half of each group were provided with a running wheel to enable voluntary exercise (C2ex, H2ex, H2ex, n = 10–12). Measurements included food intake, blood pressure and glucose tolerance. At week 14, brain, heart, muscle and fat were collected for mRNA measurement of markers for appetite regulation, cardiovascular and glucose and lipid homeostasis. Plasma leptin, insulin, triglycerides (TG), adiponectin and nonesterified fatty acid (NEFA) were determined. HC offspring weighed 12% more than CC offspring (P < 0.05). They had increased fat mass, plasma leptin and adiponectin (P < 0.05; HC vs CC), which were exaggerated by postnatal HFD (HH vs HC; P < 0.01). HFD consumption also increased plasma TG and NEFA with a doubling of food intake and 37% increase in body weight (HH vs HC; P < 0.01). Distance travelled on running wheel did not differ across groups. While exercise had no obvious effect in offspring of lean mothers consuming a low fat diet.

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075 MICROGLIA ARE ACTIVATED IN THE HYPOTHALAMUS FOLLOWING MYOCARDIAL INFARCTION

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Following a myocardial infarction (MI), inflammatory cytokines are elevated in the brain, as well as in plasma, indicating that inflammation is occurring in the brain in addition to the periphery. Microglia are the immune cells in the central nervous system and can produce cytokines when they are activated by an insult or injury. In the present study, we investigated whether MI in rats induces activation of microglia in the brain. We used immunohistochemistry to detect CD11b (clone OX-42) and morphological changes to identify activated microglia. Compared to control rats, MI-exposed rats had higher CD11b+ Iba-1+ cells in the parvocellular PVN. Activated microglia were not observed in the PVN but not in the adjacent hypothalamus following MI. The activated microglia may contribute to the result of a generalised inflammatory reaction in the brain. Echocardiography and haemodynamic parameters 2 weeks after myocardial infarction indicated reduced left ventricular function but congestive heart failure had not yet developed. In conclusion, microglia are activated in the PVN but not in the adjacent hypothalamus following myocardial infarction. The activated microglia may contribute to the increased local production of pro-inflammatory cytokines observed in the PVN after myocardial infarction and resulting reduced left ventricular function.

076 LOST THERAPEUTIC BENEFIT (LTB) IN HIGH RISK PATIENTS MANAGED FOR HYPERTENSION IN AUSTRALIAN GENERAL PRACTICE

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Background: Hypertension is a common risk factor for cardiovascular disease and despite the wide spread use of anti-hypertensive medication, many people remain uncontrolled (Blood Pressure (BP) > 130/80 mmHg). Lost Therapeutic Benefit (LTB) receiving medication without attaining target BP levels may lead to increased morbidity and mortality due to cardiovascular disease.

Aim: To estimate the extent of LTB in patients at high risk of atherothrombotic events and to model the impact of attaining target BP levels in LTB patients on cardiovascular event rates over a two year period.

Methods: The Australian REACH registry consists of 2872 high risk patients of which 2856 (99.4%) were followed for cardiovascular events over a two year period. The mean age was 72.8 ± 8.9 yrs, 65.1% were male and 78.7% had a history of hypertension. LTB was calculated as the proportion of patients receiving antihypertensive therapy who were not attaining guideline BP control targets. A hypothetical intervention to lower blood pressure to the normal range was applied to those individuals identified with LTB, to estimate the number of cardiovascular disease events (cardiovascular disease death, non-fatal stroke and non-fatal MI) which could be prevented. Logistic regression was used to find the prediction of LTB among different LTB rates for controlled versus uncontrolled and males versus females were compared using Chi squared tests.

Results: Among the 2856 Australian REACH participants, 70.1% (n = 2002) had uncontrolled blood pressure (>130/80 mmHg) and 88.3% (2522) had been taking antihypertensive medication. LTB was 70.7% (1784). The major univariate predictors of LTB were gender, age, diabetes, hypertension, carotid plaque, cholesterol, BMI and congestive heart failure. The cardiovascular event rate in those people with LTB was 5.6% while those on medication and controlled BP rate was 2.0%. Assuming a hypothetical blood pressure reduction intervention is applied to the LTB group resulting in controlled blood pressure (<130/80 mmHg), 8 cardiac events (CV death, non-fatal stroke, and non-fatal MI) per 1000 people and 21 cardiovascular disease events including coronary heart disease intervention such as CABG, coronary angioplasty, carotid surgery, etc. per 1000 people could be prevented.

Discussion: Improving BP control in patients receiving antihypertensive medication may prevent 8 cardiovascular events per 1000 people and 21 CVD events per 1000 people within this study group. At a population level, this would represent a major cardiovascular event reduction strategy.

077 THE POWER OF THE MORNING BLOOD PRESSURE SURGE AND ITS RELATION TO LONG-TERM SURVIVAL IN THE 2ND AUSTRALIAN NATIONAL BLOOD PRESSURE STUDY (ANBP2)

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Objectives: Cardiovascular risk is greatest in the morning period. We defined a new measure of the morning blood pressure surge which is derived from ambulatory blood pressure monitoring (ABPM) by the product of the rate of morning rise (ROP) and the amplitude (day night difference) giving an effective “Power” of the blood pressure rise (BPPower). We have examined the association of morning BP power and heart rate and long term clinical outcomes in elderly hypertensive patients monitored by ambulatory blood pressure monitoring in ANBP2.

Methods: BP-Power was calculated using a double logistic fitting procedure from 712 ambulatory recordings from male and female subjects. Five-year follow-up (short-term) was conducted by study nurses and 10 year follow-up was conducted via record linkage with the National Death Index with mail and telephone follow-up of survivors. Cox-proportional hazards models were used to determine the association of BP-Power on short- and long-term survival.

Results: Forty-two and 130 (18.3%) deaths accumulated over a medium- and long-term follow-up of 4.1 and 9.2 years respectively. After adjusting for age and sex, systolic BP power in the lowest quartile was associated with a 42% greater long-term risk of death in comparison to the highest quartile (OR 1.35; 95% CI: 0.82 – 2.23; P = 0.23). Diastolic BP Power had no impact on survival (OR 1.03; 95%CI 0.63 – 1.68; P = 0.90) However for heart rate power the reverse was observed. Subjects with the lowest heart rate power had a 23% lower risk of death (OR 0.77; 95%CI 0.48 – 1.46; P = 0.30).

Discussion: These findings support the hypothesis that blood pressure power and heart rate may be markers for survival outcomes in elderly hypertensive patients.

078 IS CATHERETER-BASED RENAL DENERVATION ASSOCIATED WITH A SUSTAINED BLOOD PRESSURE REDUCTION IN PATIENTS WITH RESISTANT HYPERTENSION? COMPLETE 12 MONTHS SAFETY AND Efficacy RESULTS

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Hypertension is a global public health problem of major magnitude; an estimated 30–40% of the adult population in the developed world suffers from this condition. Despite the availability of pharmacologic therapies, only half of treated patients are controlled to established targets. Renal sympathetic hyperactivity is seminal in the progression of hypertension. Catheter-based renal denervation has recently shown promise in treating this target, and thus lowering blood pressure (BP). Renal denervation using a radiofrequency catheter (Symplicity Catheter System, Ardian Inc., Palo Alto, CA, USA) was tested in patients with resistant hypertension (systolic BP ≥160mmHg on ≥3 anti-hypertensive medications, including a diuretic), Office BP and safety data were acquired at baseline and at 1, 3, 6, 9 and 12 months post-procedure. Fifty patients were enrolled at 5 centers in Australia and the EU; 5 patients were excluded for anatomic ineligibility pre-procedure. Among treated patients, mean age was 58 ± 9 yrs, 44% were female, 31% diabetic and 22% had coronary artery disease. Baseline office BP was 170/110 ± 20/15 mmHg despite patients being on a mean of 4.7 anti-hypertensive medications. Baseline eGFR was 81 ± 23 mL/min/1.73m² and heart rate was 72 ± 11/min. A preliminary analysis of this study was published in the Lancet (2009;373:1275–1281) demonstrating a substantial and sustained reduction in BP following the procedure. However, only 9 of these patients were followed up to 12 months post-procedure. This is of considerable relevance given the theoretical potential for the BP-lowering benefits of the procedure to wear off over time due to the potential of re-growth of ablated nerve fibers.
Therefore, full 12-month safety and effectiveness data is critical to ascertain the net benefit of catheter-based renal denervation. This late-breaking presentation will report on full 12-month safety, BP-lowering efficacy and supportive mechanistic data on the complete patient cohort. This should provide definitive data on whether the BP-lowering benefits initially observed are sustained over the full 12-months of the study.

RENALASE PLASMA LEVELS ARE ASSOCIATED WITH SYSTOLIC BLOOD PRESSURE IN PATIENTS WITH RESISTANT HYPERTENSION

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Evidence from experimental studies suggest that renalase, a soluble FAD-dependent protein, is involved in blood pressure regulation, possibly via degradation of catecholamines including noradrenaline. To investigate whether renalase is associated with blood pressure levels and/or indices of noradrenaline disposition in humans we studied a cohort of 22 patients with resistant hypertension (at least 3 antihypertensive drugs including a diuretic) and 4 healthy, normotensive control subjects. Radioisotopic dilution methodology and arterial blood sampling was applied to measure whole body noradrenaline (NA) spillover. Arterial plasma levels of renalase were measured by Western blot analysis using a monoclonal anti-renalase antibody and quantified using a gel documentation system (Bio-Rad Quantity One Software). Split half analysis of the hypertensive cohort according to systolic blood pressure levels (mean: 168 ± 22 vs 156 ± 9 mmHg; P < 0.001) revealed that mean arterial renalase levels were substantially lower in the patients with higher systolic blood pressure (r = 0.31 vs 125 ± 82 arbitrary units; P < 0.05), whereas whole body NA spillover tended to be higher in the group with higher systolic blood pressure without reaching statistical significance (465 ± 445 vs 407 ± 156 ng/min; P = 0.12). Arterial renalase levels were higher (238 ± 174 arbitrary units) and whole body NA spillover was lower (168 ± 78ng/min) in the normotensive control subjects (mean systolic blood pressure: 123 ± 7mmHg; P < 0.05). Correlation analysis revealed an inverse relationship between arterial renalase plasma levels and systolic blood pressure for the entire cohort (r = -0.52; P = 0.05). These data suggest that arterial plasma levels of renalase are inversely associated with systolic blood pressure in a cohort of patients with resistant hypertension. Whether this relationship is in part be explained by alleviated degradation of noradrenaline or whether alternative pathways are involved requires further investigation.

MEASUREMENT OF BLOOD PRESSURE DURING A SINGLE BOUT OF LOW INTENSITY EXERCISE IDENTIFIES PATIENTS WITH MASKED HYPERTENSION

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Masked hypertension (MH) is an independent predictor of cardiovascular mortality, but cannot be diagnosed from blood pressure (BP) taken in the clinic. We sought to determine if MH could be identified from BP or pressure waveform analysis (PWA) either at rest or during a single bout of low intensity exercise. Brachial and estimated central BP (by PWA; SphygmoCor) were recorded at rest and during -10 minutes of light intensity cycling exercise (60–70% of age-predicted maximal heart rate) in 77 untreated subjects with a hypertensive response to exercise (HRE) (aged 54 ± 8 years) and 61 patients with hypertension (HT) receiving angiotensin converting enzyme inhibitors (ACEI). All patients underwent assessment of aortic and brachial arterial stiffness via pulse wave velocity (PWV) in addition to 24 hour ambulatory BP monitoring (24ABPM). MH was defined as clinic systolic BP (SBP) <140mmHg and 24ABPM SBP ≥ 130mmHg. There were 44 (58%) HRE and 32 (42%) HT patients with MH. For the HRE group at rest, there were no significant differences between MH and normotensive subjects in any haemodynamic variable except brachial systolic BP which was higher in MH subjects (127 ± 9 vs. 120 ± 8 mmHg; P = 0.05). After correction for resting SBP, MH subjects had significantly higher brachial (167 ± 22 vs. 168 ± 15 mmHg; P = 0.05) and central SBP (154 ± 17 ± 141 ± 12 mmHg; P = 0.05) during exercise, with greater changes in both from baseline (P = 0.05). No differences were observed in the HT group. A binary logistic regression model was created and revealed sex, aortic PWV and light exercise brachial BP as significant predictors of MH. The model had high positive predictive value (95%) for detecting MH and accounted for between 49% and 69% of the variance in MH. Light intensity exercise brachial SBP was independently associated with MH, and if ≥ 175mmHg, identified MH with 75% sensitivity and 67% specificity (P < 0.001). MH could be identified in untreated individuals from low intensity exercise brachial BP but not resting PWA. Further research on the diagnostic value of BP during early phases of exercise stress testing is needed.

A SIGNIFICANT DECLINE IN INSULIN-LIKE GROWTH FACTOR-1 OF YOUNG AFRICANS PREDISPOSE THEM TO SUBSEQUENT CARDIOMETABOLIC VULNERABILITY

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Low serum insulin-like growth factor-1 (IGF-1) is an independent risk factor for cardiovascular disease and diabetes. These noncommunicable diseases are extremely common in urban black Africans. This cross-sectional study involved 211 African and 316 Caucasian men and women (aged 20–70 yrs). Fasting glucose, insulin, lipids, albumin, creatinine, liver enzymes, cotinine, high-sensitivity C-reactive protein (hsCRP), reactive oxygen species (ROS), IGF-1, blood pressure (BP) and pulse wave velocity (PWV) were determined.

IGF-1 was lower in the Africans (P < 0.001), and in both ethnicities IGF-1 declined significantly when comparing age quartiles. But in African men and women IGF-1 declined significantly from age quartile 1 to 2 (r = -0.65, P < 0.001 for both), not seen in young Caucasian men and women (r = -0.08, P = 0.45; r = -0.10, P = 0.34). This was confirmed after adjustment for BP, insulin resistance, hsCRP, cotinine, γ-glutamyl transferase and ROS. Only young Africans showed significant negative correlations of IGF-1 with BP, PW and HDL-cholesterol. To conclude, Africans presented lower IGF-1 levels than Caucasians due to an accelerated decline in serum IGF-1 concentration prior to 40 years of age. Strong associations of low serum IGF-1 with arterial stiffness in young Africans suggest that the loss of cardiometabolic protection by IGF-1 could predispose them to earlier disease onset.

SPIRONOLACTONE IMPROVES LIGHT EXERCISE CENTRAL SYSTOLIC LOADING IN SUBJECTS WITH EXAGGERATED EXERCISE BRACHIAL BLOOD PRESSURE: A RANDOMISED CONTROLLED STUDY

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Exaggerated exercise blood pressure (EEBP) predicts mortality. The mechanisms of this association are unknown but have been linked to increased central arterial stiffness and raised central blood pressure (BP) during low-stress physical activity. Spironolactone has antifibrotic effects that may reduce large artery stiffness and lower exercise central BP. This study aimed to test these hypotheses. Untreated patients without hypertension or coronary artery disease, but with EEBP (N = 112; aged 55 ±6 years, 58% male), were randomized to 3 months spironolactone (25 mg daily; n = 57) or placebo (n = 55). An EEBP was defined as brachial BP ≥ 190/105 (mmHg) in men, and ≥ 210/105 mmHg (men) during maximal exercise. Arterial stiffness was recorded by aortic pulse wave velocity (PWV). Brachial BP was estimated by sphygmonanometer and central BP by radial tonometry at rest, during low-stress physical activity (cycle ergometry at 60–70% maximal heart rate) and after maximal exercise. Patients also underwent 24 hour ambulatory BP monitoring (24ABPM). V0_max testing and 2D echocardiography for left ventricular (LV) structure and function. At baseline, aortic PWV was associated with peak exercise systolic BP (SBP) (r = 0.24; P = 0.01), low-stress central pulse pressure (r = 0.23; P < 0.03) and VO_max (r = -0.26; P = 0.003). Compared with placebo, spironolactone significantly reduced 24ABPM SBP (−3.8 ± 7.3 versus 1.0 ± 8.7 mmHg; P = 0.004), maximal exercise brachial SBP (−8.3 ± 16.3 versus −0.5 ± 11.0 mmHg; P = 0.002) and maximal central SBP (−7.6 ± 11.6 versus −1.3 ± 10.5 mmHg; P = 0.007) but did not change aortic PWV, VO_max or LV parameters (P > 0.05 for all). Moreover, central SBP (−5.4 ± 12.1 versus 0.7 ± 13.1 mmHg; P = 0.03) and the systolic pressure-time integral (−230 ± 477 versus −111 ± 419 mmHg) were significantly reduced during low-stress exercise (P < 0.05 for both), whereas low-stress brachial SBP was unchanged by spironolactone (−3.8 ± 16.0 versus 1.3 ± 13.8 mmHg; P = 0.14). We concluded that maximal exercise BP, as well as submaximal central systolic BP during light activity, are improved by spironolactone in patients with EEBP, but these changes cannot be attributed to reduced central artery stiffness.

IMPROVED QUALITY OF LIFE FOLLOWING UNILATERAL LAPAROSCOPIC ADRENALECTOMY IN PATIENTS WITH UNILATERAL PRIMARY ALDOSTERONISM

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For patients with unilateral primary aldosteronism (PAL), unilateral laparoscopic adrenalectomy (ADX) corrects biochemical and leads to cure or improvement in hypertension control. While most studies have focused on clinical and biochemical outcomes, to our knowledge there are no data on the effects of ADX on quality of life (QOL). In the current study, QOL was evaluated...
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Tumoral necrosis factor α (TNF-α) has been implicated in PE, the result of which mediates the placental dysfunction, vascular remodeling, and synthesis of pro-inflammatory cytokines. The production of TNF-α is increased in the placenta of women with PE compared to normal pregnant women. However, the exact mechanism by which TNF-α contributes to the development of PE is still unclear. This study aimed to investigate whether TNF-α levels are increased in the plasma of pregnant baboons with PE and to determine the potential role of TNF-α in the pathogenesis of PE.

Methodology:

We have previously reported that TNF-α infusion increases blood pressure, induces proteinuria and increases soluble FMS-like tyrosine kinase 1 (sFlt-1) plasma concentrations in pregnant baboons. The aim of the current study was to determine if the source of increased circulating sFlt-1 in this model of preeclampsia (PE) is the placenta, and to determine if changes in TNF-α levels are associated with changes in sFlt-1 levels in pregnant baboons.

Results:

In the current study, we found that TNF-α levels were significantly increased in the plasma of pregnant baboons with PE compared to control baboons. The increase in TNF-α levels was associated with an increase in sFlt-1 levels, suggesting that TNF-α may be involved in the pathogenesis of PE by promoting the release of sFlt-1 from the placenta.

Conclusion:

These results provide further evidence for the role of TNF-α in the pathogenesis of PE and highlight the importance of targeting TNF-α as a potential therapeutic strategy for the treatment of this disease.

References:


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THE RECRUITMENT OF BLOOD-BORNE QDOT-LABELED CELLS INTO ATHEROSCLEROTIC PLAQUE

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We have come full circle in determining whether bone marrow or bone marrow precursor cells can enter the diseased vessel wall to form new endothelial or smooth muscle cells. Thus, we sought to determine whether blood-borne cells could be recruited during an atherogenic diet. Rabbits were injected for four days with 15µg/kg/day GCSF and on the fifth day, CD45+ bone-borne cells (characteristic of stromal lineages) were isolated, labeled with QDots, and stored at −70°C. Then, the same rabbits were fed and atherogenic diet for 4 weeks and 10th QDot-labeled autologous cells were injected weekly during this time. Viability of cells in vivo was confirmed the day after using flow cytometry. Labeling peptide, Ang 1−7, the ascending aorta and coronary artery (LMA) were then studied by confocal microscopy. QDot-positive ‘neo’ endothelial cells were visible in both the ascending aorta and the LMA, which appeared to be concentrated on the shoulder of the plaque in the LMA. However, QDot-positive neo-intimal cells and medial cells were only found in the LMA and not the ascending aorta. There were also QDot-negative cells throughout both vessels. Conclusions: Blood borne CD45-negative cells form part of the neo-endothelial layer during an atherogenic diet, but only form neo-intimal and medial cells in the LMCA at this time point. These studies strongly support the hypothesis that blood born cells can be recruited into atherosclerotic plaque, and that they appear to be concentrated in the ‘shoulder’ region of plaque.

HEART RATE-DEPENDENCE OF AORTIC PULSE WAVE VELOCITY IN RATS

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Aortic pulse wave velocity (aPWV), a surrogate measure of arterial stiffness, is a strong independent predictor of cardiovascular disease and all-cause mortality. Whilst studies have shown associated changes in arterial stiffness with acute changes in heart rate (HR), the effect of HR on aPWV at different mean arterial pressures (MAPs) have not yet been fully investigated. In this study, aPWV was measured in age-matched (12 weeks), urethane-anesthetised normotensive rats (n = 6) at different HRs across a wide MAP range (40–160 mmHg). HR changes were achieved by atrial pacing at randomly sequenced rates (350, 400 and 450 bpm) after the resting HR was lowered with multiple doses of a bradycardic agent (zatebradine, 4.2 mg/kg/hr). MAP changes were achieved by infusions of phenylephrine and sodium nitroprusside respectively (30 µg/min/hr). Effects of HR on aPWV were assessed at each low (40–80 mmHg), medium (80–120 mmHg) and high (120–160 mmHg) MAP range. Data are presented as mean ± se. To establish a HR-dependency of aPWV, ANOVA was applied to compare means of aPWV at two different HRs within the same MAP range. HR is shown to have no significant effects on aPWV at the low and medium MAP ranges, but a significant difference in mean aPWV was observed in the high MAP range between the lowest and highest HRs. This finding indicates a HR-dependence of aPWV at high MAPs in rats. This is of particular interest as studies in humans have shown conflicting results of HR effects on arterial stiffness when blood pressure was relatively unchanged. These results suggest that the values of MAP and HR may also need to be accounted for when making comparisons across studies.

ATHERO-PROTECTIVE EFFECTS OF THE ACE INHIBITOR, PERINDOPRIL, ARE PARTIALLY MEDIATED VIA ACTIVATION OF BOTH MAS AND AT2 RECEPTORS IN APOPOLIPOPROTEIN E-DEFICIENT MICE

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It is now well established that ACE inhibitors mediate athero-protective effects, both independently and via their actions on the renin-angiotensin-aldosterone system. It is also well recognized that cardiovascular disease can result from defects of components of the contractile apparatus. Recently, utilizing cardiac-specific transgenic mice with a constitutively active (ca) mutant of phosphoinositide 3-kinase [PI3K(p110α)] it was demonstrated that caPI3K(p110α) has a protective role in a setting of myocardial infarction (MI), in contrast, mice expressing a dominant negative (dn) mutant of PI3K developed heart failure more rapidly in response to MI than control mice. Thus, activation of the PI3K signalling pathway proves to be a potential therapeutic strategy for the treatment of heart failure; but the underlying molecular mechanisms remain elusive. Considering the range of cardiomyopathies that have been attributed to mutations of genes coding for structural proteins of cardiac muscle, the aim of this study was to identify associations between the protective effect of active PI3K(p110α) and cardiac structural components. Here we have adapted a 3D Virtual Muscle (VMus3D) model to cardiac muscle that represents the location of these structural proteins for visualising micromyaaray data obtained from ventricles of non-infarcted (sham) and MI operated caPI3K and dnPI3K transgenic mice (relative to non-transgenic). Using VMus3D for pattern recognition, a criterion was established that enabled us to select expression profiles that affected cardiac muscle structure according to PI3K perturbation. Our approach has enabled us to draw on the entire gene expression dataset from a different perspective unbiased by highly differentially expressed transcripts. This has enabled us to identify mechanisms linking PI3K to cardiac muscle structure regulation and glue homoeostasis. In particular, we have identified mRNAs regulated by PI3K perturbation under basal conditions that code for components associated with muscle structure at the z-disc and costamere (Dag1, Ankrd23, Rock2, Cx43, and Cx45). We propose that the combined effects of these components may determine the heart’s ability to appropriately respond to mechanical stress in a setting of heart failure and provide a possible explanation and potential model (2-disc: costamere: hypertrophy axis) for how this could be operating.

INCREASED LEFT VENTRICULAR MASS AND DECREASED LEFT VENTRICULAR RELAXATION IN TYPE 2 DIABETES MELLITUS WITH RESISTANT HYPERTENSION

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Resistant hypertension (HTN) is defined as blood pressure (BP) that remains above goal in spite of concurrent use of 3 antihypertensives medications (AHT) of different classes. According to the current guidelines the BP goal of type 2 diabetes mellitus (T2DM) is <130/80. The prevalence of resistant HTN and its effect on cardiac function in T2DM is not well documented. We hypothesized that resistant HTN in association with LV hypertrophy is common in this population and is associated with left ventricular (LV) structural and functional abnormalities. Data was examined from subjects with T2DM (n = 1214) attending a diabetes complication and assessment program at a single tertiary hospital who had a transthoracic echocardiogram (TTE) and blood pressure measurement. A clinical history was taken and supine brachial BP measured. The mean age was 64±12 years. 72% of the male, mean duration of diabetes mellitus (DM) 12.8±4.7 years, mean number of AHT 1.6±1.3 and mean body mass index was 31.6±6.4 kg/m². Subjects were divided into 4 groups: Groups A and B were nonresistant (BP <130/80) and in both AHT (n = 75, 6.2%); subjects in Group C were at goal BP of <130/80 and were on <3 AHT (n = 75; 58.1%), and Group D subjects were classified as resistant HTN i.e. not at BP goal and were on ≥3 AHT (n = 239, 19.7%). Group D had higher LV mass index and lower mitral annular early diastolic velocity (marker of LV relaxation) compared to the other groups (Figure 1). A significantly greater proportion of subjects in Group D had LV diastolic dysfunction when compared to Group C (22.1% (P<0.01)). Our findings indicate that resistant HTN in T2DM is common and is associated with an increased LV mass and decreased LV relaxation.
### 093

**KYNURENINE IS A NOVEL ENDOTHELIUM-DERIVED VASCULAR RELAXING FACTOR IN ATHEROSCLEROSIS**

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Indoleamine 2,3-dioxygenase (IDO) is a heme-containing enzyme that metabolizes the essential amino acid tryptophan to kynurenine. In acute inflammatory diseases, including malaria and sepsis, IDO is induced and contributes to hypotension. We investigated whether IDO plays a role in the regulation of vascular tone in atherosclerosis, a disease associated with chronic inflammation. The expression of IDO protein was determined in atherosclerotic lesions of Apoe–/– single and Apoe–/– Ido–/– double gene knockout mice, and in human carotid plaques by immuno-histochemistry. Synthesis by pressure was recorded in conscious mice using a computerized, non-invasive tail cuff system. Vessel function was assessed in vitro using a standard myograph system. IDO was expressed in atherosclerotic lesions of Apoe–/– mice and humans, whereas the protein was absent in non-diseased arteries or the arteries from Apoe–/– Ido–/– mice. Pharmacological inhibition of IDO by its competitive inhibitor 1-methyltryptophan increased blood pressure in Apoe–/– but not in Apoe–/– Ido–/– mice. In the myograph system, addition of tryptophan caused a relaxation in the pre-constricted aortic rings from Apoe–/– but not Apoe–/– Ido–/– mice. Also, kynurenine relaxed aortic rings in an endothelium-independent manner, whereas other known kynurenine pathway metabolites had no material effect on vessel relaxation. Arterial relaxation by Kyn was mediated by activation of the adenylylate and soluble guanylate cyclase pathways. This study suggests that tryptophan metabolism to kynurenine may contribute to the regulation of vascular tone in atherosclerosis, opening the possibility for novel treatments of ischemic complications arising from atherosclerosis.

### 094

**COST BENEFIT OF SALT REDUCTION TO COMPLEMENT EXISTING CLINICAL HYPERTENSION PROGRAMS**

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Excess dietary salt is a key contributor to high blood pressure and vascular disease. National and international organisations recommend that average population salt intakes are reduced to below 6g per day The objective of this study was to compare the likely costs and benefits of adding a national salt reduction program to existing clinically based disease prevention strategies. We undertook a search for studies reporting the actual or projected costs and/or effects of hypertension management and salt reduction in Australia, summarised the data and compared the costs and effects of the two strategies. We found that hypertension management in Australia costs ~$1 billion a year (~$500 million on drug therapies and $500 million on other costs) and likely prevents approximately 10% of the disease burden attributable to high blood pressure. A national salt reduction strategy to reduce average daily intakes from 6.8g to 5g in Australia was projected to cost ~$15 million dollars a year, (1–2% of the hypertension program). This would reduce population blood pressure levels by 3–4mmHg, resulting in a 15–20% reduction in premature vascular disease in the immediate term and a 20–30% reduction in the longer term, due to the cumulative effects of the attenuation of the population rise in blood pressure with age. The costs of the salt reduction program are anticipated to decrease over time. Expansion of hypertension recognition and treatment would also prevent more events but would be less cost effective. In conclusion, there appears to be a strong case for the adoption of a nationally coordinated salt reduction program to the existing clinical hypertension control program with substantial health benefits accrued at minimal additional cost.

### 095

**AROTIC STIFFNESS, BUT NOT CENTRAL OR BRACHIAL BLOOD PRESSURES, PREDICT PHYSICAL QUALITY OF LIFE**

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Unless severe and uncontrolled, hypertension is commonly asymptomatic. However, some studies suggest that lower brachial blood pressure (BP) in patients with uncomplicated hypertension may improve quality of life (QOL). The relation between central BP and QOL has never been assessed, but may be relevant because large differences in central systolic BP (SBP) can occur between individuals with similar brachial SBP. This discrepancy between central and brachial SBP is mostly attributed to individual variation in large artery stiffness. We sought to determine the relation between QOL BP (brachial and central) and large artery stiffness in 104 patients receiving therapy for uncomplicated essential hypertension (aged 63 ± 8 years, 53% male) who were free from a history of cardiovascular or renal disease. The SF-36 health survey was used to quantify QOL. Brachial BP was assessed in the clinic (while seated and standing), at home (7-day average) and by 24-hour ambulatory monitoring (24ABPM). Clinic central BP and aortic pulse wave velocity (PWV) (for arterial stiffness) were estimated by applanation tonometry. Neither brachial nor central BP's were associated with QOL measures (P > 0.05 for all). However, physical functioning scores significantly declined across tertiles of aortic PWV (tertile 1: 89 ± 14, tertile 2: 76 ± 22, tertile 3: 76 ± 29) even after correcting for age, gender, clinic brachial SBP and 24ABPM SBP (ANCOVA P = 0.028). On multiple regression analysis, aortic PWV (but no BP measure) independently correlated with physical functioning (β = −0.26, P = 0.012), but only accounted for 6% of the variance in this QOL component. We conclude that physical well being is negatively associated with large central artery stiffness, which provides further evidence that interventions to reduce arterial stiffness may improve patient outcomes. Furthermore, central BP appears to offer no additional information beyond brachial BP regarding QOL. Whether this finding will be supported by the use of a hypertension-specific QOL survey needs to be assessed.

### 096

**ANALOG STUDIES OF CHANGES OF ARTERIAL PRESSURE WAVEFORM WITH AGING**

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Changes of features in cardiovascular parameters occur in different periods of life for populations. These changes can be investigated by means of physiological modeling, such as technique of multi-branched models to mimic the human arterial system. The aim of this study was to simulate the influence of aging on the shape of radial arterial pressure waveform. An aorta-radial transmission line model of the left arm was constructed and consisted of 11 segments from ascending aorta to radial artery through aortic arch, left subclavian, axillary and brachial branch. Each segment was constructed by one electrical circuit representing viscous properties of blood (Ri), inertial properties of blood (L), compliant properties of arteries (C) and leaking through small branches (RL). For simulation of increasing age, only Young’s modulus (E) of the conduit arteries was increased in a range of 10% while wall viscoelasticity (ηw) was set no change. Radial arterial pressure waveform was obtained from an aortic pressure wave input in proximal and transfer function between them were calculated by means of FFT analysis. With increases in Young’s modulus, from 11.1 × 106 dyn/cm2 to 15.5 × 106 dyn/cm2, the radial arterial waveform showed an earlier wave (wave velocity increase) and more sharp in ejection duration (compliance decrease) but no distinct change in systolic (SD, ~0.2 mmHg) and diastolic (SD, ~0.1 mmHg) pressure values. The peak value of modulus of transfer function moved toward high frequency and became less as a result of viscous attenuation of wall viscosity. In this study, an electrical analog model reflecting properties of vasculature has been constructed. The technique can be used to accurately simulate the realistic changes in arterial pressure waveform.

### 097

**EDHF RESPONSES IN MUSCLE RESISTANCE ARTERIES FROM TYPE-1 DIABETIC RATS**

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The endothelium-derived relaxing factors, endothelin-derived hyperpolarizing factor (EDHF) and nitric oxide (NO), can invoke endothelium-dependent hyperpolarisation and relaxation of the vascular smooth muscle. This study investigates the effects of 10-week streptozotocin (STZ)-induced type 1 diabetes on endothelial function in the cremaster artery, a skeletal muscle resistance artery, compared to corresponding control rats. In MOPS (3-(N-morpholino)propanesulfonic acid)-buffered Krebs buffer containing 10 μM indomethacin, endothelium function was assessed using pressure myography by measurement of the internal diameter of isolated segments of cannulated cremaster arteries, maintained at a physiological pressure of
70 mmHg. Blood glucose was significantly greater (P<0.05, Student’s t-test) in diabetic rats (33.0±0.2 mM, n=22) than in control rats (11.9±0.5 mM, n=20). Acetylcholine (ACH)-induced vasodilation, but not levocarnitamol (L)-induced vasodilation, was significantly attenuated (P<0.05 ANOVA) in diabetic rats (Cmax 87.7±1.5% maximum relaxation; EC50 118 nM, 95% CI 77–178 nM; n=18) compared to control rats (Cmax 96.8±0.9%, EC50 53 nM, 41–88 nM; n=17). Inhibition of the nitric oxide (NO) pathway using l-nitro K-arginine methyl ester (l-NAME, 100 μM) and 1H-[1,2,4]oxadiazolo[4,3-a]quinazolin-1-one (ODQ, 1 μM), did not significantly change ACh responses (ANOVA, P<0.05) in either group of rats. Remaining relaxations were blocked by the addition of the small- and intermediate/large-conductance Ca2+-activated K+ channel inhibitors, apamin (1 μM) and charybdotoxin (0.1 μM), respectively. Inhibition of the EDHF pathway with apamin (1 μM) together with the intermediate-conductance Ca2+-activated K+ channel inhibitor, TRAM-34 (1 μM), significantly impaired ACh-mediated relaxations in both diabetic and control rats. The residual response was abolished by the addition of the large-conductance Ca2+-activated K+ channel inhibitor iberiotoxin (0.1 μM). The present study indicates that in type-1 diabetes, there is endothelial dysfunction in cremaster arteries. The findings also suggest that ACh-mediated relaxations are mediated by EDHF, and involve small-, intermediate- and large-conductance Ca2+-activated K+ channels.

FENOFIBRATE EXAGGERATED ACTH-INDUCED HYPERTENSION IN RATS

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In the present study, we investigated the effect of fenofibrate on glucocorticoid-induced hypertension in rats. Male Sprague-Dawley rats were treated with saline, adrenocorticotropic hormone (ACTH) (0.2 mg/kg/day) or dexamethasone (0.03 mg/rat/day) s.c. for 13 days. Fenofibrate (100 mg/kg/day per rat) was mixed in ground food. Systolic blood pressure (SBP) was measured by the tail-cuff method. Aortic superoxide production was measured by lucigenin-enhanced chemiluminescence. Thymus wet weight was measured as a marker of glucocorticoid activity and plasma NO2- concentrations or aortic superoxide production. The expression of CYP2C23, CYP2C11 or CYP4A was not affected by fenofibrate, ACTH or dexamethasone. In conclusion, fenofibrate exaggerated ACTH- but not dexamethasone-induced hypertension. The effect of fenofibrate on ACTH-induced hypertension was independent of 20-HETE production.

ANALYSIS OF BIOCHEMICAL CHANGES IN THE HEARTS OF ADULT INTRAUTERINE GROWTH RESTRICTED OFFSPRING USING FTIR IMAGING MICROSPECTROSCOPY

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Growth restriction in utero as a result of maternal malnutrition has been linked with gender specific decline in cardiac performance during adulthood. Here we examined the effect of growth restriction in rats, due to maternal low protein diet (LPD), on the changes in fibrosis and biochemical composition of the heart in the female offspring in adulthood. Wistar Kyoto (WKY) dams were administered either LPD (6.7% casein) during pregnancy and lactation or normal protein diet (NPD; 20% casein). At 14 weeks of age, hypertension was induced through a 4 week continuous infusion of angiotensin II (ANGII, 200ng/kg/min) in female NPD and LPD offspring (N=7) via subcutaneous minipumps while control group received saline. Arterial blood pressure was measured using a tail cuff method. At 18 weeks of age the offspring were perfused fixed and organs collected. The hearts were weighed, sliced and the heart volumes stereologically determined according to the Cavalieri method. Alternate left ventricle (LV) slices from normotensive and hypertensive offspring were used for assessment with Fourier transform infrared (FTIR) imaging micro-spectroscopy. FTIR images were processed using Unsupervised Hierarchical Cluster Analysis. Birth weights of the LPD offspring were significantly lower compared to NPD (6.5±0.3 g and 7.6±0.3 g, respectively), however in adulthood (18 weeks of age) this difference was no longer significant. ANGII infusion resulted in lower body weight in NPD and LPD offspring (P<0.0002). Absolute heart and LV volumes were both significantly lower in LPD offspring. Relative heart and LV volumes were significantly increased by ANGII administration. Perivascular fibrosis and both media to lumen and adventitia to lumen ratios were increased in the NPD hearts only (P<0.0009 and P<0.004, respectively). FTIR images recorded from the LV indicated significant differences in collagen distribution and density between NPD and LPD hearts that is attributed to collagen disorder in the LPD hearts. The LPD hearts had lower intensity amide-I band but overall higher optical density in the mid infrared. FTIR imaging spectroscopy shows promise as an independent modality for examining changes in the macromolecular chemistry of the adult IUGR heart.