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PHOTOSTIMULATION OF CHANNELRHODOPSIN2-TRANSFECTED C1 NEURONS ACTIVATES PERIPHERAL SYMPATHETIC VASOMOTOR AFFERENT 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 retropertioneal nucleus (1:1 ratio of C1: non-C1 neurons, 70% of all C1 neurons). Pulsed photostimulation of the rVLM with 473 nm laser light (400 pulses, 20 Hz, 30 min) increased arterial 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 sSNND (P<0.05; N=6). Single LL pulses produced a massive evoked burst of sSNND (peak; 1443±223% relative to baseline, onset latency; 28±1 ms; N=9) followed by a longer-lasting reduction of sSNND (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 sympathoexcitatory 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.

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 AT, receptor have been isolated, and products consequently formulated. In addition, certain polyphenolics and flavanols have been shown to reduce BP via mechanisms involving ACE and/or the vasculature. We assessed the effects of chlorogenic acid (CGA), a polyphenol found in coffee, on BP in the adult SHR over a 48 h period, following administration of a single oral dose. Compared to saline control, CGA caused both dose- and time-dependent reductions in BP over the 48 h monitoring period. CGA at 75 mg/kg lowered BP (P<0.05 or better) at 3, 6, 9 and 24 h post administration. At 250 mg/kg, lower BP was still evident at the 48 h time point (237±1.4 vs 223±2.0 mmHg, P<0.01). The greatest reduction in BP was observed at the 3 h (18–20%; 75 mg/kg and 125 mg/kg) and 6hr (21%; 250 mg/kg) time points. In comparison, Enalapril (1 mg/kg) reduced BP in the 3–9 h time window (8–12% vs control), with BP values returning to baseline by the 24 h point. In isolated aortic rings pre-contracted with phenylephrine (PE, 1 µM) CGA caused dose-dependent relaxation, however in the PE pre-contracted perfused-mesenteric vascular bed, CGA exerted no relaxation but instead resulted in a paradoxical increase in contraction. In summary, the acute BP model is an appropriate tool to evaluate the potential antihypertensive effects of bioactives. Results also suggest that BP lowering actions of CGA are likely to be mediated via mechanisms other than the lowering of peripheral vascular resistance.

AMPLIFICATION 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 application tonometry. We set systolic peak and diastolic nadir of each to cuff brachial systolic (SP) and diastolic (DP) pressure. We then measured the carotid waveform by application tonometry, and calibrated this by assuming that mean (MP) and DP were the same in the arm.

Studies were undertaken in 70 (27 women) volunteers and patients (mean age 70 years SD 14.8) 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 needing 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. Application of the brachial artery against bone could not be ensured through the bipapal apnoea measurement. High values of FF for the brachial and carotid artery were similar to those of Asklepious 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.

ESTIMATION OF AORTIC FLOW FROM DERIVED AORTIC PRESSURE WAVEFORMS: A MODELLING STUDY

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Measures of aortic flow velocity and cardiac output have been estimated from arterial pressure waveforms, but never before (to our knowledge) from the transfer functions (TF) that link radial artery pressure to aortic pressure waveforms, and aortic pressure to aortic flow waveforms. Such a process can be used (when recent 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 apices 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 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 waveform 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⁻³).

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 84 cm/s (calculated) c.f. 107 cm/s measured by Doppler; stroke volume averaging 86 ml (calculated) c.f. 81 ml (measured by Doppler), and cardiac output averaging 3.7 L/m (calculated) c.f. 5.1 L/m (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 1992:20:952] 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 (Form Factor (FF)) of systolic pressure (PP) [Hypertension 2009;54:414]. This study was undertaken to establish whether FF values were identical in central and peripheral vessels. It was also determined what proportion of PP was used. The value 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), ejection duration (ED), PP amplification (PPA), brachial PP and aortic augmentation index (AIX). The database [JAHSH 2008;2:28] comprised 8621 observations in 1505.

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 and aortic FF and age, MP, HR, ED, PPA, PP and AIX.

<table>
<thead>
<tr>
<th>Mean (mmHg)</th>
<th>SD</th>
<th>R² for correlation</th>
<th>Regression p-value</th>
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</thead>
<tbody>
<tr>
<td>FF (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Radial</td>
<td>33.6</td>
<td>4.4</td>
<td>0.004</td>
</tr>
<tr>
<td>Age (y)</td>
<td>69.4</td>
<td>13.2</td>
<td>0.004</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>66.6</td>
<td>12.7</td>
<td>0.003</td>
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<tr>
<td>ED (msec)</td>
<td>318.1</td>
<td>32.4</td>
<td>0.077</td>
</tr>
<tr>
<td>MP (mmHg)</td>
<td>95.3</td>
<td>13.3</td>
<td>0.263</td>
</tr>
<tr>
<td>PPA (%)</td>
<td>21.7</td>
<td>9.0</td>
<td>0.027</td>
</tr>
<tr>
<td>AIX (%)</td>
<td>27.1</td>
<td>11.9</td>
<td>0.334</td>
</tr>
</tbody>
</table>
| PP (mmHg)   | 66.6 | 17.9              | 0.000             | 0.005 | <.03

Wide variation in radial artery FF results in substantially different values of MP being calculated if a set formula such as diastolic + FF % of PP is used. The value of radial 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 an ω-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 included identical amounts of protein (15% from torula yeast 30% w/w), carbohydrate (53% w/w), fat (7% w/w), AIN-93G vitamins and minerals except for Se. The diets were either sufficient (Se 124.1 μg/g diet) or deficient (Se 1.3 μg/g diet). Heart rate, body weight, food and water intake were not different between groups. Thus, it would appear that Se, due to its antioxidant actions, can ameliorate the hypertensive effects of ω-3 fatty acid deficiency.

IS THE PHASE OF THE MENSTRUAL CYCLE IMPORTANT WHEN SCREENING FOR PRIMARY ALODSTERONISM (PAL) IN WOMEN, AND DOES RENIN ASSAY METHOD MATTER?

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The most popular screening test for PAL is the plasma aldosterone (aldo)/renin ratio (ARR), Measurements, dietary Na+, posture and time of day all affect renin and aldosterone levels, and can result in false -ve or +ve ARR if not controlled. Because fluctuations in both estrogen (E2), raised renin substrate but direct renin concentration (DRC) fails to prevent increases in angiotension (A-II) and progesterone (prog, aldo-antagonist) effect aldosterone and renin levels, we studied effects of phases of the menstrual cycle on ARR, measuring renin as both DRC and plasma renin activity (PRA, a better surrogate for A-II). Normotensive, non-medicated volunteers (n=19) had blood and urine collected midmorning for measurement of plasma aldolo (by HPLC-tandem mass spectrometry), DRC, PRA, serum E2, prog, LH and FSH and urinary aldosterone and creatinine at 0 and 24 hours (1 SDl) after starting the last menstrual period. Cmparisons with follicular phase (day (10) levels, luteal (day (20) levels of prog were much higher [follicular median 0.5 range 0.3-7.6] vs luteal 39.8 (12.1-75.1) mmol/L, P<0.001) and FSH levels lower [4.9 (3.4-35.9) vs 2.6 (1.3-15.1) IU/L, P<0.001], while E2 [389 (202-820) vs 263 (104-777) ng/ml, P<0.001] and progesterone levels were also higher (P<0.001). LH [3.4 (2.4-6.9) vs 1.3 (0.7-11.7) IU/L, NS] were non-significantly lower. Luteal levels were higher than follicular for plasma aldolo [170 (133-524) vs 454 (181-1141) mmol/L, P<0.001] and urinary aldoo [105 (26-334) vs 233 (166-551) pmol/mmol creatinine, P<0.001]. DRC [28 (10-58) vs 38 (15-78) μ IU/L, P<0.01], PRA [2.1 (1.0-5.7) vs 3.1 (1.6-9.2) ng/ml/hr, P<0.001], and ARR calculated using DRC [8.3 (2.3-49.9) vs 14.2 (2.3-69.7), P<0.001] but not when calculated using PRA [109 [24-227] vs 133 (30-300), NS]. In two subjects luteal ARR was elevated when calculated using DRC but normal using PRA.

EFFECTS OF BLOOD PRESSURE LEVELS AT PRESENTATION AND ACHIEVED IN THE FIRST 24 HOURS ON HAEMATOMA GROWTH IN ACUTE INTRACRANIAL 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 analysed for volume. We investigated whether effects of BP levels on absolute and proportional haematoma growth 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 randomisation 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), 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 support 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 rather than 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 blood pressure reduction via inhibition of ROS production. The aim of this study was to examine ROS formation and the expression of the different NOX isoforms NOX1, 2 and 4 in aortae of aged spontaneously hypertensive rats, a model of endothelial dysfunction and oxidative stress. ROS formation was measured by dihydroethidium (DHE) stain of tissue sections or by lucigenin chemiluminescence of aortic homogenates. NOX expression was assessed by immunoblotting and a calculation of NOX proteins by immunofluorescence. Endothelial function was assessed by aortic ring relaxation response to acetylcholine. Aortic sections of SHR showed a 5.7±1.3 increase in ROS formation, compared to aged matched WKY controls (P<0.01). This was inhibited by the NOX inhibitors diphenylene iodonium, apocynin and the novel inhibitor VAS2870. In contrast, eNOS inhibition with L-NAME or by xanthine oxidase inhibition with oxypurinol did not decrease ROS levels. NOX1 and NOX2 were upregulated in SHR aorta compared to WKY rat aorta (3.4±0.6; P<0.01 and 1.6±0.1; P<0.01, respectively), whereas NOX4 expression remained unchamped. In tissue sections NOX1 showed strong positive staining in the intima of SHR, whereas it co-localized with an endothelial cell marker. NOX1 staining was only weakly positive in the aortae of WKY. NOX2 distribution was similar in both rat strains. Aortic endothelial function, as indicated by the maximal relaxation response to acetylcholine, was significantly impaired in SHR versus WKY rats (SHR: 56.2±2.6% vs. 80.8±2.2%, respectively) resulting in similar mean blood pressure of both groups (SHR 141±1.5 vs. 151±2.1 mmHg, and 83±0.3 vs. 86±0.4 mmHg, respectively). These data suggest that impaired NOX1 expression in SHR aorta as is expression of NOX1 and NOX2. Importantly, the ectopic expression of NOX1 in endothelial cells appears to affect vascular function in a NADPH oxidase inhibitor-reversible manner. NOX1 may thus represent a novel target for the treatment of hypertension.

SHORT TERM FAT FEEDING ALTERS PLASMA CHOLESTEROL ESTER AND CERAMIDE PROFILES IN NEW ZEALAND WHITE RABBITS

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Although the association between obesity and hypertension is well known, the underlying mechanism remains elusive. Previously, we have shown that 3 week fat feeding in rabbits results in a greater vasoconstrictor mass, hypertension, tachycardia and elevated renal sympathetic nerve activity. Because dyslipidaemia is an independent cardiovascular risk factor and has been associated with hypertension we now wish to compare plasma lipid profiles in male New Zealand White rabbits fed a normal fat diet (ND 3.5% fat, n=11) or high fat diet (HFD 13.5% fat, n=11) for 3 weeks. Plasma lipids were extracted by a modification of the Folch method. Concentrations of diacylglyceride (DAG), triglyceride (TAG), ceramide and cholesterol esters (CE) were obtained after analysis by liquid chromatography mass spectrometry. We measured 6 species of ceramide, 22 of DAG, 43 of TAG and 29 of CE. HFD rabbits exhibited greater plasma concentration of total monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) in the CE from the six ceramide species were significantly increased in HFD compared with ND rabbits. Ten of the 29 CEs examined were significantly elevated in HFD compared with ND rabbits; 16 and 20 carbon fatty acids and free cholesterol predominated this group of elevated species. Concentrations of total DAG (P=0.004) and TAG (P=0.04) did not differ between dietary groups. Only one DAG (16:0, 20:4, P=0.04), and one TAG (18:1, 18:1, 20:4, P=0.02) were elevated in HFD compared with ND fed rabbits. Elevated ceramide and CE concentration is consistent with fat feeding and an increase in white fat pad mass and if continued uninterrupted, may lead to atherosclerosis and vascular dysfunction. The finding that DAG and TAG species were largely unaffected is surprising but may indicate sequestration of fatty acids in white adipose tissue at this stage of the fat feeding regimen. This model may prove useful to further characterise the relationship between plasma lipids and hypertension.

CYTOCHROME P450 EPoxyGENASE PRODUCTS OF ARACHIDONIC ACID ARE ALTERED IN THE METABOLIC SYNDROME

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Metabolism of arachidonic acid (AA) by the cytochrome P450 (CYP450) epoxygenase leads to formation of four epoxyeicosatrienoic acid (EET) regioisomers. EETs produce vascular relaxation and hence represent a novel target for blood pressure reduction via inhibition of ROS formation. Diacylglycerols (DAG) and fatty acid monoethanols were previously shown to be metabolites of AA by CYP450 and DHETs (Dihydroxyeicosatrienoic acids) are formed by soluble epoxide hydrolase (sEH). We have previously shown that metabolism of arachidonic acid by CYP450 ω-hydroxylase results in increased plasma and urinary 20-HETE in the metabolic syndrome. This study aimed to compare CYP450 epoxygenase metabolites of AA in fat-fed and DEF animals, and male and female Sprague-Dawley rats, in a case control study of untreated men and postmenopausal women with features of the metabolic syndrome (MetS). Volunteers were recruited from the general population with cases (n=16) and controls (n=18) matched for age and gender. EETS and DHETs were measured in plasma and platelets after base hydrolysis, by gas chromatography mass spectrometry. The volunteers were aged 55.9±1.5 (MetS) and 54.5±1.5 (controls) with blood pressure and BMI of 135/97±2.7/1.6 mmHg and 34.3±3.2/1.5 mmHg respectively (MetS), and 112/69±2.1/1.8 mmHg, P=0.007. Plasma DHETs were not different between the groups 11.6±0.95 ng/ml (MetS) compared with 11.5±1.06 ng/ml (controls). In contrast, plasma platelet EETs were significantly reduced in the MetS (1.4±1.0 ng/10⁹ cells) compared with controls (2.1±0.3 ng/10⁹ cells), P=0.04. Platelet DHETs were not different between the groups. In conclusion, our results suggest that increased formation of EETs may represent a novel target for blood pressure reduction via inhibition of ROS formation in the metabolic syndrome.

HYPERTENSION INDUCED BY ω-3 FATTY ACID DEFICIENCY: THE ROLE OF THE RENIN-ANGIOTENSIN SYSTEM

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Deficiency of ω-3 fatty acids has been demonstrated to induce hypertension in the rat. However, the aetiology of the elevated blood pressure remains unknown. In these studies, the role of the Renin-Angiotensin System (RAS) in the hypertension caused by life-long deficiency of dietary ω-3 fatty acids was examined. To achieve this, a low dose of angiotensin converting enzyme (ACE) inhibitor (perindopril, 0.3mg/kg) was chronically administered (for 4-weeks) to 36-week-old animals that were life-long deficient (DEF) or sufficient (SUF) in ω-3 fatty acids; blood pressure was measured using tail cuff sphygmomanometry. In a further study, hypothyroidic RAS gene expression changes were analysed in DEF and SUF animals prior to 3-months-old, and following (9-months-old), the development of hypertension. Administration of perindopril reduced the hypertension (SBP and DBP) in DEF animals, without affecting the blood pressure of SUF animals, see Table Regarding gene expression, at 3-months AT1α was no longer over-expressed. Overall, these findings demonstrate the involvement of the RAS and Angiotensin system in maintaining physiological blood pressure levels, with reduced hypertensive response of the RAS in DEF animals that are deficient in ω-3 fatty acids.

P(S/A) DEF(D) SUF(P) F (d)

SBP (mmHg) 126±6.1 146±5.3±3±4 122±6.7 129±5.2

DBP (mmHg) 100±3.8 111±4.3±3±4 99±3.4 104±4.0

P-perindopril treatment; significant differences are indicated by superscript letters.
CVD IN INDIGENOUS AUSTRALIANS: OPPORTUNITIES FOR IMPROVING OUTCOMES ACROSS THE CONTINUUM OF CARE

A Brown, Centre for Indigenous Vascular and Diabetes Research, Baker IDI Heart and Diabetes Institute, Alice Springs, NT, Australia

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 relating to the disparity experienced by Indigenous Australians, Cardiovacular 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 normal care for Indigenous Australians, in rural/remote areas and in metropolitan settings. National data has identified poor outcomes and under use 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, burden, provision of care, experience of services, adverse outcomes and their determinants, and opportunities for reform within the management of CVD among Aboriginal Australians.

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CVD IN INDIGENOUS AUSTRALIANS: OPPORTUNITIES FOR IMPROVING OUTCOMES ACROSS THE CONTINUUM OF CARE

A Brown, Centre for Indigenous Vascular and Diabetes Research, Baker IDI Heart and Diabetes Institute, Alice Springs, NT, Australia

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 relating to the disparity experienced by Indigenous Australians, Cardiovacular 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.

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associated with night-time active periods in the Schlager (PvL/Ju) hypertensive mice leading to hypertension, and an exaggerated circadian day-night difference in BP.

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

PJ Davern, MJ McKinley, Baker IDI Heart & Diabetes Institute, Melbourne; 2Howard Florey Institute, Melbourne

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 forebrain. 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 were killed 1-3 wk recovery period. C-Fos immunochemistry 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 vehicle infused controls. This is the first report of direct neural projections arising from pressor responsive neurons in discrete LPB subnuclei innervating the MnPO and CeAm. Although, there was no evidence for ascending projections from neurons activated by hypertension 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.

**FLOW AND ENERGY LOSS IN SACULAR ANEURYSMS ARISING FROM STRAIGHT AND CURVED PARENT ARTERIES - HAEMODYNAMIC ANALYSIS FOR ASSESSING RISK OF Rupture**

A Farnoux, Y Gian, M Morgan, A Avolio, The Australian School of Advanced Medicine, Macquarie University, Sydney, Australia

Saccular aneurysms 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 saccular aneurysms 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 those 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 aneurysms, 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**

JL Favario, H Al Sadoun, MD Linden, School of Medical Sciences, RMIT University, Victoria

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 ischemic 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 10 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 initiation of 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, a novel solv flow cytometry, was unaffected and the structure and function of the platelet fibrinogen receptor GPIb-lla, 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 sequestration of important signalling molecules such as ATP, ADP, adrenaline, ionomic calcium and serotonin. 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 thrombus affecting 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 antiplatelet therapy.
HOMOCYSTEINE AND DNA DAMAGE GENETIC POLYMORPHISMS ARE ASSOCIATED WITH HYPERTENSION IN PREGNANCY

D Furness, S Thompson, R Nowak, V Zhang, G Dekker, C Roberts, Robinson Institute, Research Centre for Reproductive Health, University of Adelaide, South Australia

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 including 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 analysis. 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 (MTHFR) 2756 G alleles were associated with PE + SGA compared to controls (P = 0.043, resp). Polymorphisms within the methylenetetrahydrofolate reductase (MTR) GR genotype was associated with PE + SGA compared to healthy pregnancies (P = 0.030). Neonatal NAT1 T allele connected for age and BMI was associated with maternal mean arterial pressure and sBP (P = 0.011, P = 0.001). MTR and MTHFR 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-acylation of aromatic amine and hydrazine drugs. Polymorphisms in NAT1 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 NAT1 polymorphisms and maternal mean arterial pressure and sBP at 15 weeks gestation. These indicate that fetal/placental metabolic enzymes may contribute to raised maternal blood pressure and that those associated with increased homocysteine may be linked with preeclampsia, a common hypertensive disorder in pregnancy.

ASCORENENTION OF CARDIAC FUNCTION WITH TRAN-SORHISOCARDIOGRAPHY 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 and is of key importance to cardiovascular function. The intensity of sunlight is the major source of vitamin D. There is an increasing prevalence of vitamin D deficiency in many populations worldwide, 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 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. At 14 weeks of age non-invasive trans-thoracic echocardiography was performed in female offspring (n = 10 control and n = 8/group). The hearts were then subjected to 20 minutes ischemia and 11/2 hours reperfusion. At the end of the reperfusion period the left ventricle was sliced and incubated in 1% 2, 3, 5 triphenyl tetrazolium solution (T2S), 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. Disregulation of coronary flow and the extent of vascularity may be factors which contribute to the increased susceptibility to ischemia/reperfusion injury.

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

M Glover, KM O'Shaughnessy, University of Cambridge, Cambridge, United Kingdom

Dissecting the molecular regulation of renal sodium transport by the thiazide-sensitive NCC has provided important insights into the mechanisms underlying hypertension. NCC regulation is orchestrated by a scaffold of proteins including SPAK and WNK4 kinases. Intuitively such dynamic regulation must also involve phosphatases. Two distinct phosphorylation controlled regulatory pathways for NCC exist; Type 1 mediated by WKAR affecting trafficking to the surface membrane and Type 2 involving modulation of intrinsin transporters kinetics by phosphorylation of N-terminus of NCC. In this study the role of the kinase inhibitors 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 NCC T58D/A, kinase-dead PP4 and PP4 and WNK4 726I. Xenopus oocytes were then injected with cRNA from these constructs and NCC expression followed using 2Na+ flux and confocal microscopy. PP4 expression inhibited NCC activity by 68% without affecting surface membrane expression. The effect was specific to PP4 and dependent on both its phosphatase activity and NCC-T58b to accept the phosphate. Phosphatases did not affect WKAR regulation of NCC membrane trafficking. In addition, site mutation of WNK4 726A enhanced fifteen fold its susceptibility to the kinase inhibitors SB202190 and SB203580.

Thus NCC activity but not trafficking is regulated by PP4 with clear implications for blood pressure control. Furthermore, single site mutation of WNK4 increase significantly its sensitivity to established kinase inhibitors increasing the prospects for pharmacological intervention 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 have been shown to arise because of pressure gradient which exists between the intracranial and intracranial compartments. Invasive studies have demonstrated that the higher systolic to diasystolic ratio of pressure in the intracranial space compared that in the intracranial space is the origin of this pressure gradient. In intracranial hypertension, cerebrospinal fluid pressure (CSF) pulsatility increases along with mean CSF pressure and as it approaches the intracranial pulse pressure (IOP) the intravascular pressure gradient decreases respectively, leading to cessations of pulsations. Characterizing interaction between ocular and intracranial space pressure in humans involve difficult and limited surgical procedure. Therefore in this study we propose 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. Intracranial hypertension is simulated by increasing the CSF outflow resistance or a decrease in intracranial compliance. The retinal venous pressure is obtained as a function of the difference between IOP and CSF. 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: Vr = -0.1095*CSFp + 1.6164*CSFp - 2.9658.

Which RvP is the Retinal Venuous Pulsatile. Results also show a decrease in IOP 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 intracranial and intracranial space. The model may allow a non-invasive approach to cerebrospinal fluid pressure estimation.
SYNTHESIS OF RENALASE, A POTENTIAL NOVEL MONOAMINE OXIDASE, IS NOT LIMITED TO RENAL AND CARDIAC TISSUE

SC Hennebry, N Ekelund, F Socratous, G Desir, G Lambert, N Szrajczer

MP Schlaich

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In human tissues catecholamine disposition is regulated by two classes of membrane-bound enzymes: monoamine oxidase (MAO) and vascular adhesion protein (VAP-1). Recently, a novel enzyme called renalase with MAO, we hypothesised that the distribution of renalase in human tissues would not be restricted to renal and cardiac tissues. Human tissue samples were obtained from the Victorian Institute of Forensic Medicine (VIFM) Tissue Donor Bank at autopsy. The informed consent of the donor’s next-of-kin was obtained before the autopsy. All protocols were approved by the VFM Ethics review Committee. Total RNA and soluble protein were extracted from these tissues. RT-PCR and western blotting were performed to determine the evidence of renalase gene expression. Our results demonstrate that renalase has a far larger representation in human tissues than previously reported and is not restricted to the kidneys and heart. Western blotting showed the presence of renalase in regions of the kidney and peripheral nervous systems, in adrenal glands, adipose tissue, and peripheral blood mononuclear cells. RT-PCR was used to validate these observations and enable the identification of several splice variants of the renalase transcript. These splice variants appear to be tissue-specific and point to a functional diversity of renalase function. Further investigations into the structural and functional characteristics of renalase may help to facilitate our ability to diagnose and treat disorders involving an imbalance in the levels of monooamine neurotransmitters. We are currently pursuing investigations into the regulation of renalase gene expression using a variety of cell culture and animal models.
genes of the hypothalamic axis, in particular NR2C2, IGF1R, IGF2R, PCSK2, leptin, leptin receptor and PPARy 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 urinary 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 (7.40) mmol/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=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=–0.49 (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, an Australian population sample, most participants were consuming excessive amounts of sodium. Dietary sodium and the Na/K ratio were both significant predictors of BP. These results indicate that a population wide reduction in dietary sodium would be effective in reducing blood pressure in Australia.

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

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Pituitary adenylate cyclase activating polypeptide (PACAP) is an excitatory neuropeptide with known central and peripheral cardiovascular effects. Intrathecal (IT) administration of PACAP increases splanchic sympathetic nerve activity (sSNA) and HR, but surprisingly elicits no change in MAP. Additionally, it is unknown if PACAP is involved in either the tonic or reflex control of blood pressure, at the level of the spinal cord. Moreover, no pharmacological studies have been conducted which of the three PACAP receptors (PAC1, VPAC1 and VPAC2) are responsible for the actions of PACAP at the spinal cord. This study sought to determine if 1) PACAP has a role in the tonic control of blood pressure at the level of the spinal cord, and 2) to determine pharmacologically if the PACAP effects previously reported are mediated via the PAC1 receptor.

Urethane anaesthetised (3g/kg) n=10 adult male Sprague-Dawley rats (n=12) were vagotomised, ventilated and paralysed. MAP, HR (derived from ECG) and end tidal CO2 were recorded and responses were recorded for 60 mins. PACAP(6-38), following pretreatment with PACAP(6-38), were significantly attenuated compared to PACAP-38, showing a similar duration of BP surge (1.7–0.4 h) compared to the Schipper BP 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 BP 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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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 milfarsartan has been combined with various other pharmacophores (selenium, phenol and ebselen) with the aim that they will have dual actions. The AT1 receptor antagonist potency of these compounds has been previously confirmed, with all of the antioxidant-substituted milfarsartan analogues, except ebselen-milfarsartan, showing antagonist properties in rat isolated right atria. 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-amidinopropyl) hydrochloride)-induced haemolysis assay (mouse C57/B6 isolated red blood cells) in the presence and absence of 1 mM glutathione (GSH). AAPH results in free radical-mediated haemolysis which is quantitated by calculating the area under the absorbance curve. In the absence of GSH neither the antioxidant groups nor milfarsartan analogues protected against haemolysis. However in the presence of GSH, ebselen, selencyclost, phenol and phenol-substituted milfarsartan protected against lysis (N=4 each; P<0.05 vs. vehicle), whereas the other milfarsartan analogues did not. For comparison, the antioxidant capacity of these compounds was also tested in a tissue-based preparation (mouse C2/B6 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 quercitin (10, 30 and 100 µM). In this assay ebselen and phenol but not selencyclost (10 µM) each protected against doxorubicin-induced negative inotropy. The substituted milfarsartan analogues did not protect against oxidative damage, however pre-treating with milfarsartan, phenol-milfarsartan was able to protect against radical-mediated damage (N=4 each; P<0.05 vs. vehicle). The data from these experiments show ebselen-substituted milfarsartan to longer remain a potential antioxidant property that ebselen displays nor the antagonist potency milfarsartan possesses. In contrast, the phenol-substituted milfarsartan retains AT1 receptor antagonist potency but not antioxidant properties in the tissue-based assay as receptor binding interacts with the antioxidant moiety. Altering the structure of this interaction may or may not limit this interaction may result in a dual action drug with therapeutic potential for the disease atherosclerosis.

DYNAMIC SYNCHROTRON 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 consequent decline in heart function. Conventional angiography is not adequate to assess impairments in micro-vascular function. Using synchrotron imaging we are now able to detect small vessel calibres (<40 µm) using a conventional X-ray device) and quantify the rate of change in variables during dark light cycle. The diurnal range in BP and heart rate were 153% and 68% greater respectively in BPH compared to BPN normotensive mice (P<0.05 vs. vehicle). However, the rate of rise in BP from inactive to active period is constant between the two strains (8.7±2.0 and 8.4±1.9 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 h, 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 h) compared to the Schipper BP 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 BP normotensive counterparts. The mechanisms that regulate the timing of these changes may be key to the understanding the cause of the hypertension in these mice.
in disease models. These findings further indicate that in the early diabetic state there is already localised coroan endothelial dysfunction.

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

BK Kemp-Harper, Department of Pharmacology, Monash University, Melbourne

The therapeutic utility of the NO/cGMP pathway has long been recognised with nitrovasodilators such as glyceryl trinitrate (GTN) used for the treatment of cardiovascular disorders such as angina, hypertension and heart failure for >100 years. The clinical efficacy of traditional NO donor treatments is limited, however, by susceptibility to scavenging by superoxide (O2-) and tolerance development, properties which confound the treatment of heart failure. Importantly, we have also demonstrated that NO donors are vasoprotective, with potent vasorelaxant and anti-platelet effects and an ability to limit vascular oxidative stress. Thus in rat, mouse and human arteries, NO donors such as Angeli’s salt and IPA/NO target predominantly the soluble guanylyl cyclase (sGC)/cGMP signaling pathway to mediate vasorelaxation. Moreover, in contrast to GTN, NO donors do not develop vascular tolerance. Recently, our studies have shown that NO donors limit oxidative stress, lowering vascular NADPH oxidase-derived O2- production in isolated intracranial arteries by ~65%. Such an effect is resistant to S-nitrosylation, ODO and absent in NO2 deficient mice. This suggests that NO donors modulate NAD(P)H containing NADPH oxidase possibly via a direct, cGMP-independent action. In addition, we have provided the first evidence that the vasoprotective actions of NO donors are preserved in disease with the ability of NO donors to induce vasorelaxation and inhibit platelet aggregation sustained in the setting of hypertension (SHR), diabetes and hypercholesterolemia (apoE–/– mice). In conclusion, NO donors with their favourable vasoprotective properties, lack of tolerance development and preserved bioavailability under conditions of oxidative stress, offer considerable advantages over traditional nitrovasodilators 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-isoform 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, C57BL/6) and arginase II knockout (ArgIIKO) mice were mounted in myographs and cumulative concentration-response curves (CRCs) to the NO donors, glycyl trinitrate (GTN) or sodium nitroprusside (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 < 0.05) and SNP (n = 6, P < 0.05), while in ArgIIKO aorta it was not (P = 0.1). GTN-tolerant vessels 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 < 0.1). 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.

EFFECT ON TIME DELAY BETWEEN PRESSURE AND FLOW SIGNALS ON CALCULATION OF CRITICAL CLOSING PRESSURE AND RESISTANCE 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 methodology of estimating cerebral CCP and RAP. Calculations were performed on sequential cardiac cycles (N = 27) with radiofrequency records 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 time derivative of the cerebral blood flow waveform and backwards by 10, 20, 30, 40, 50 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 vitro, in vivo 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 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 AP. 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) hypertension caused by high doses of dilators, which limited autoregulatory capacity. Both confound assessment of the structural TPR-amplifier. Its magnitude is substantial, relatively uniform in different beds and greater than in essential and SHR hypertension. Its magnitude is best estimated during NHB, when it was 1.84 ± 0.05 without a significant change in heart rate or cardiac output. Ghrelin infusion resulted in a marked increase in muscle sympathetic nervous activity (MSNA), measured by microurography (from 18.2 ± 20.4 bursts per min, P < 0.005) while no change occurred during saline infusion (from 18.2 ± 27 ± 1 bursts per min). Ghrelin, but not saline, induced a rise in plasma glucose concentration (from 4.4 ± 1.0 to 4.8 ± 0.1 mmol/l, P < 0.05). A stress test consisting of 5-min of forced mental arithmetic was performed following the infusion of saline and ghrelin. During saline, stress induced a significant change in mean arterial pressure (+19 mmHg), heart rate (+21 bpm) and MSNA (+35%, P < 0.05). During ghrelin infusion, the changes in heart rate were less pronounced (±16 bpm, P < 0.05, compared with saline). Changes in blood pressure and MSNA during the ghrelin infusion were slightly but not significantly reduced 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 vasoileatory effect rather than by affecting the central nervous system. Furthermore, ghrelin may contribute to the stress induced cardiovascular responses.

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 receptor activation mediated cardioprotective effect of MR blockade. Spironolactone (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 markers, active caspase-3, acinus, and anti-apoptotic protein, ARC were measured. Apoptosis mediates the cardioprotective effect of MR blockade. Spironolactone (100 nM) and 1 μM reduced infarct size (mean values of 37 ± 2.2%, N = 9 and 36 ± 2.2%, 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 × 10^6 ± 0.9, N = 7 versus 2.2 × 10^6 ± 0.3, N = 7, P < 0.05) and restored
by 10 mM spironolactone (1.9 × 10^{-6} to 0.2, N = 6). Conclusion: Spironolactone acts merely by excluding corticosteroids from mineralocorticoid receptors, but as a protective inverse agonist at low concentration and by activation of anti-aphyotic protein(s).

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 compete with n-3 PUFAs for common metabolic enzymes, and during incorporation into plasma lipid fractions. This has the potential to increase the atherogenic index of the n-6:n-3 ratio. Therefore, there is a need to ascertain the exact nature of that mechanism.

CENTRAL INFUSION OF RENIN INHIBITOR ALISKIREN PREVENTS SYMPATHETIC HYPERACTIVITY AND HYPERTENSION IN DAHL SALT-SENSITIVE RATS ON HIGH SALT

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The brain renin-angiotensin system is a major player in salt-induced hypertension. Chronic central blockade of AT1 receptors prevents sympathetic hyperactivity and hypertension in Dahl salt-sensitive (S) rats on high salt. In the present study, we examined whether renin produced in the brain contributes to the salt-sensitive hypertension. A preliminary dose-response study showed that intra-cerebroventricular (icv) infusion of renin inhibitor aliskiren (0.2 mg/kg/d) and 1 mg/kg/day similarly attenuated salt-induced hypertension without visible adverse effects in Dahl S rats on high salt (8% NaCl, HNa). In the main study, Dahl S rats on HNa or regular salt (0.6% NaCl, RNa) were infused icv with aliskiren (0.2 mg/kg/d) or artificial cerebrospinal fluid (aCSF) as control via osmotic minipumps. One group of Dahl S rats on high salt received subcutaneous (ic) infusion of aliskiren at the same rate. After 2 weeks of infusions, BP, HR and sympathetic nerve activity (RNSA) at rest and in response to air, as well as arterial baroreflex function in response to acute changes in BP by iv phenylephrine or nitroprusside were assessed in conscious rats.

Data are means ± SEM (n = 4–9, *P < 0.05, vs RNa; a: P < 0.05, vs HNa; a: sc aliskiren or ic acSF). These results indicate that centrally administered aliskiren indeed prevents sympathetic hyperactivity and hypertension in Dahl S rats on high salt, and demonstrates that brain renin is essential for salt-induced sympathetic hyperactivity and hypertension.

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 molecular 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 protein. Angiotensin (pro)renin can generate AngI 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 protein, (P(RR), AGT, ACE1, ACE2, AT1R, AT2R, MAS1, amion, 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 before labour from pregnancies that carried a female fetus (P=0.06). After labour, there was a significant decrease in decidual protein mRNA in pregnancies carrying a female fetus (P=0.035). (P(RR) mRNA was highest in placenta (P=0.004). AGT 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=0.02). AT1R mRNA was highest in placenta (P=0.001). AT2R and MAS1 receptor mRNAs were not detected. It is concluded that in amniotic fluid 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, ACE1 protein is localized to fetal capillary endothelial cells and AT1R mRNA and protein are present, and syncytiotrophoblast may contain 2 independent RAS pathways, one in fetal vessels and one in syncytiotrophoblasts. 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.

OPTIMISING BLOOD PRESSURE ASSESSMENT IN THE OBSTETRIC DAY ASSESSMENT UNIT

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Obstetric day assessment units (DAU) are used to assess maternal blood pressure (BP) during pregnancy. This method of BP monitoring began to reduce the morbidity associated with hospital admissions. A wide variety of protocols are in use for assessing maternal BP in the DAU. It remains unclear what the optimal time period is, for observation in the DAU. We measured the BP (non-invasively) in 40 pregnant women in the DAU. Women (n=40) had their BP measured at multiple time points (11 time points) over 4 hours. An analysis of repeated measures was undertaken to assess the change in the average BP over time. Multiple comparisons (aired T-tests) underwent a Bonferroni correction. Data are presented as the mean ± sem. Maternal systolic BP (SBP) altered significantly over the period of observation (P<0.006). Women with a diagnosis of a hypertensive disorder of pregnancy (HDP) had SBP changes similar to those women without hypertension. The average SBP fell significantly (P<0.001) from baseline (137.7±2.0 mmHg) until the nitude at 30 minutes of rest (130.7±1.7 mmHg). Diastolic BP (DBP) did not statistically change in women with or without a HDP. The average SBP after 1 hour of observation, if the first BP reading was discarded, did not significantly differ from the average SBP after 2, 3 or 4 hours of observation. Inclusion of the baseline SBP in the hourly averages results in significant differences between the 1st and 2nd (P=0.012) and 3rd and 4th (P=0.003) hours of observation. These results demonstrate that the day assessment period could be reduced from 4 hours to 1 hour. The initial BP reading should be discarded and blood pressures taken every 15 minutes for a total of 3 readings.

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 supporting 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 post-stroke. We have also identified genetic and oxidative signalling factors involved in this response in rats. Functional deficits in behaviour were detected between 1 and 7 days post-stroke (P<0.001). 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±3% and 23±7%) respectively, compared to contralateral brain regions (P<0.05). Double immunofluorescence labelling revealed that the marked increase in blood vessel numbers 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 expression increased between the cortex and the border zone at 7 and 14 days post-stroke (up to 19 fold P<0.005) 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 repertusion 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 calorie 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 20–45%. 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 (ACEI). 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 75.24±12.97 ml/min to 86.37±11.15 ml/min (n=19, P<0.04). Comparison of the ARB and ACEI groups after treatment indicated that GFR was higher in the ARB group than in the ACEI 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 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 10² 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 0.5: ± 0.011%, P = 0.04) and 3.4-fold (11.1: ± 1.9 colonies/well versus 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 correlation. These results indicate that in severe obesity there is no impairment of EPC function, implying a potential role for NGF in regulating coronary blood flow in the PR fetus.

**ABIDING – EXTENDING THE UTILITY OF OSCILLOMETRIC BLOOD PRESSURE DEVICES IN GENERAL PRACTICE**

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Peripheral arterial disease is an important contributor to Australia’s burden of disease. A simple method to objectively assess leg arterial perfusion is the Ankle-Brachial Index (ABI). 13,700 oscillometric BP devices have been distributed to Australian GPs by the HBPRCA. ABIDING seeks to expand the utility afforded by these machines by validating them for ABI determination in general practice.

Method: GPs will be recruited through the Victorian REACH database. The Australian Registry X (a prospective, observational registry to provide long-term follow-up (36 months) of patients at high risk of atherothrombotic events) consists of 2,782 participants. ABIDING is a cross-sectional validation study. Doppler (by research nurse) and oscillometric (by practice nurse) ABI measurements will be performed. The primary outcome is the correlation of ABI defined as the ratio of the highest brachial systolic BP recording to each lower limb systolic BP.

Secondary outcomes are: (i) Correlation of baseline ABI and incident adverse CVD events; (ii) Change in ABI, BMI, Edinburgh Claudication score and waist circumference from baseline.

Analyses: Descriptive analyses will be used to investigate the agreement between the two procedures. Sensitivity, specificity, positive predictive value and negative predictive values comparisons between methods for those with abnormal ABI (< 0.9) will be reported. For a sample of 340 we have 80% power to detect a mean difference of 0.05 for our primary endpoint.

Results: In recruitment phase.

Conclusions: Validation of the oscillometric determination of ABI will allow an instrument designed to identify an individual risk factor for CVD (high BP) as a diagnostic tool for generalised arteriopathy (PAD) in primary care.
Reduced Angiotensinogen Gene Expression Following Angiotensin II Stimulation of Astrocytes Expressing the Angiotensin Type 1a Receptor

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Angiotensin II (Ang) acts via AT1 receptors in several brain regions to regulate blood pressure – one site is the rostral ventrolateral medulla (RVLM) where Ang directly excites sympathetic premotor neurons. Increased angiotensin type 1A (AT1) receptor activity in astrocytes of the RVLM leads to a sustained increase in blood pressure (BP) in conscious, freely moving rats. As astrocytes produce the majority of angiotensinogen (Ao) found in the brain, we hypothesized that increased AT1 receptor activity in astrocytes might increase Ao production, leading to increased local Ang production and sympathetic activation. Positive feedback regulation of Ao production by Ang has been demonstrated in liver. Thus the aim of this project was to determine whether Ang II modulates Ao production by astrocytes. Experiments were performed in primary cultured astrocytes. Endogenous expression of AT1a receptors in cultured primary astrocytes is very low and insufficient to elicit a detectable intracellular signaling response when stimulated with AngII. We increased AT1a receptor expression by transfection with replication-deficient adenovirus expressing the receptor under control of the ubiquitous CMV promoter. This enabled detection of a robust intracellular signaling response to AngII. Using quantitative PCR we observed that in AT1a receptor transfected cells, Ao gene expression was significantly reduced by 74.7 ± 3.5% (n = 4, P < 0.005) by AngII (100nM) stimulation. This effect was blocked by co-administration of the AT1 antagonist candesartan (1μM). These novel findings do not support the original hypothesis, but indicate that the AT1a receptor couples to a negative feedback mechanism to regulate Ao production in astrocytes. Negative feedback regulation of Ao production via the AT1a receptor has also been reported in cultured cardiac fibroblasts. Further experiments will examine whether this regulation is observed in astrocytes in vivo.

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 Pharmaceutical Benefits payment records from January 2003 to December 2006. Only Concessional patients were included because many AHT products fall under the General copayment. Patients newly initiated to; Calcium Channel Blockers (CCB), Angiotensin Two Receptor Antagonists (A2RA) and Angiotensin Converting Enzyme (ACE) inhibitors were identified and the following compliance information determined: (1) number of scripts dispensed in the 12 months post initiation, (2) average time between repeats and (3) Medication Possession Ratio (MPR). More than 85,000 concessional patients were initiated on these AHT products. Assessment of compliance found the interval between repeats for AHTs combined was less than 34 days for 75% of patients and MPRs exceeded 90% (considered compliant), while the average number of scripts collected in the first year was only 8.0 per patient (considered non compliant). There were also differences between products. Mean intervals between repeats ranged from 28 (trandolapril) to 44 (captopril) days, while average scripts per year ranged from 3.8 (captopril) to 8.8 (lercanidipine). Using scripts counts over a one year period to assess patient compliance 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.
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 AT1AMUT exhibited higher BP and heart rate (HR) levels compared to controls (systolic BP AT1AMUT 134±6.5 mmHg; AT1AWT 110.5±5.8; P< 0.05; HR AT1AMUT 531±5; AT1AWT 455.5±5 beats/min; P< 0.0001). BRS was dimunition in transgenic versus wild-type littermates (AT1AMUT 1.23±0.17 ms/mmHg; AT1AWT 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 in transgenic BSH animals in the lowest gain estimation (velocity = 0.06). 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 AT1 receptors, these studies provide new evidence for a sustained control of central sympathetic vasomotor pathways and also inhibition of the cardiac baroreflex which may contribute to the hypertension.

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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 females reported five years ago revealed a substantial disparity abnormalities compared with age and sex matched normal controls, our objective was to examine the consistency and possible progression of already described disturbances in cardiovascular 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 at 80.7±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±8.6 vs 127±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 (129.3±195 vs 190.4±53.7 g; P<0.0153), LV mass index (72.4±5.8 vs 103.0±18.4 g/m2; P=0.0102) and mitral inflow deceleration time (157.5±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 thickness, 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 discuss optimal treatment for children with GSH.

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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 cardioregional 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 aldosterone-producing adenoma (APA) and whether the degree of Uprot and strength of this relationship is reduced following correction of hyperaldosteronism by unilateral adrenalectomy. Subjects with APA (n = 26) underwent measurement of Uprot and UNa in 24h urine collections before and after adrenalectomy (follow up duration 15.3 ±12.4 months). Following surgery, mean clinic blood pressure (BP) fell (from 151.7±19.8/95.8±5.3 to 134.5±14.5/83.4±30.3 mmHg; P<0.0001) in the systolic BP, but with a significant fall in number of antihypertensive medications (from 1.9±1.2 to 0.7±1.1; P<0.0001). Uprot and urinary volume decreased after adrenalectomy (from 201.2±98.3 to 108.6±37.1 mg/day; P<0.0001 and from 2464.4±854.2 to 2005.3±716.4 ml/day; P<0.0005). There was a positive correlation between Uprot and creatinine ratio and UNa/creatinine ratio both before (r=0.47, P<0.0001) and after surgery (r=0.44, P=0.0242) adrenalectomy. Unilateral adrenalectomy reduces proteinuria in patients with APA. The rapid response in some patients is consistent with a role for hyperfiltration in its etiology. A positive relationship between urinary protein and sodium excretion observed not only in the present study but also after surgery. One explanation for this persisting relationship could be long term renal exposure to hyperaldosteronism resulting in glomerular damage and an ongoing tendency to salt-sensitive protein excretion. These findings suggest that both high aldosterone levels and higher dietary salt intake could contribute to increased cardiovascular risk in these patients.

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CENTRAL GHRELIN ADMINISTRATION REDUCES ARTERIAL PRESSURE, HEART RATE AND CARdiovascular REACTIVITY TO ACUTE AIRJET STRESS IN RABBITS

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Ghrelin is predominantly produced in specific endocrine cells in the stomach and acts in the hypothalamus and brainstem to regulate pre-prandial hunger and meal initiation. More recently, ghrelin has been shown to play a role in cardiovascular regulation. In cardiovascular disease, ghrelin is elevated with improved cardiovascular function following ghrelin administration (Van Hoeijen et al. 2012). Therefore, the aim of the current study was to determine if ghrelin increased cardiovascular function. Female New-Zealand white rabbits were randomly assigned to either a control group (15 animals) or an i.c.v. ghrelin treatment group (20 animals). After 14 weeks of follow-up, rabbits were anesthetized and exposed to acute airjet stress. Administration of ghrelin increased cardiovascular function (systolic blood pressure 134.6±13.7 vs 145.4±14.9, P<0.0001) and increased diastolic blood pressure (124.8±12.7 vs 139.7±14.5, P<0.0001). Uprot and urinary volume were increased (0.0015 vs 0.0001 and 14.5±6.5 vs 20.8±5.9 ml/day, P=0.0015). A correlation analysis indicted a strong relationship between systolic blood pressure and ghrelin (r=0.38, P=0.01). In conclusion, ghrelin administration increased cardiovascular function in rabbits exposed to acute airjet stress. The present study shows that ghrelin administration results in increased cardiovascular function, which may contribute to the development of hypertension.
 term regardless of diet post-weaning. This study highlights how maternal nutrition can detrimentally impact on cardiovascular risk factors in the next generation.

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 high-fat diet (HF) or a low-fat diet (LFD). The dams were mated with males. At weaning, female offspring from C dams were fed Chow (CC) and offspring from H dams were fed Chow (HC). Half of each group were provided with a running wheel to enable voluntary exercise (Cex, HCEx, HCex, 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), adiponcin 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 adiponcin (P<0.05; HC vs CC), which were exaggerated by postnatal HFD (HC vs H; 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 exaggerated by postnatal HFD (HH vs HC; P<0.05) and decreased body weight, food intake, fat mass, plasma TG and leptin in HHEx vs HH (P<0.01). Blood pressure was elevated in offspring from obese dams consuming HFD (HH vs HC; P<0.001) but this was reversed by exercise (HHEx vs HH; P<0.05). Thus maternal obesity program adipogenesis and the rise in blood pressure by postnatal HFD. Exercise reduces the deleterious effects of maternal obesity with greater beneficial effects in offspring of obese mothers consuming HFD. Exercise had no obvious effect in offspring of lean mothers consuming a low fat diet.

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 (ROR) and the amplitude (day-night difference) giving an effective “Power” of the blood pressure rise (BPPower). We have examined the association of morning BPPower and heart rate and long term clinical outcomes in elderly hypertensive patients monitored by ambulatory blood pressure monitoring in ANBP2.

Methods: BPPower 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 BPPower 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 BPPower in the lowest quartile was associated with a 42% greater long-term risk of death in comparison to the highest quartile (HR 1.42; 95% CI: 1.10 – 1.85; P=0.006). Lowering of BPPower had no impact on survival (HR 1.03; 95%CI 0.72 – 1.48; P=0.84). Diastolic BPPower had no impact on survival (HR 1.01; 95% CI: 0.81 – 1.25; P=0.87).

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

079

RENALEAL PLASMA LEVELS ARE ASSOCIATED WITH SYSTOLIC BLOOD PRESSURE IN PATIENTS WITH RESISTANT HYPERTENSION
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Evidence from experimental studies suggest that renasle, a soluble FAD-dependent protein, is involved in blood pressure regulation, possibly via degradation of catechol-amines including noradrenaline. To investigate whether renasle 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. Radiochromat dilution methodology and arterial blood sampling was applied to measure whole blood noradrenaline (NA) spillover. Arterial plasma levels of renasle were measured by Western blot analysis using a monoclonal anti-renasle antibody and quantified using a gel documentation system (Bio-Rad Quantity One Software). Split half analysis of the renasle concentration in systolic blood pressure levels (mean: 186 ± 22 vs. 156 ± 9 mmHg; P < 0.001) revealed that mean arterial renasle levels were substantially lower in the hypertensive cohort with higher systolic blood pressure (r = 0.31 vs. 125 ± 82 arbitrary units; P < 0.005), whereas whole blood NA spillover tended to be higher in the group with higher systolic blood pressure without reaching statistical significance (465 ± 445 vs. 407 ± 195 mg/min; P = 0.12). Arterial renasle levels were higher (238 ± 174 arbitrary units) and whole blood NA spillover was lower (186 ± 78 mg/min) in the normotensive control subjects (mean systolic blood pressure: 123 ± 7.5 mmHg; P < 0.05). Correlation analysis revealed an inverse relationship between arterial renasle plasma levels and systolic blood pressure for the entire cohort (r = –0.52; P < 0.05). These data suggest that arterial plasma levels of renasle are inversely associated with systolic blood pressure in a cohort of patients with resistant hypertension. Whether this relationship is accounted for by alleviated degradation of noradrenaline or whether alternative pathways are involved requires further investigation.

080

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 wave 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 antihypertensive therapy (HRT) (aged 61 ± 7 years). All subjects 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) ≤ 140 mmHg and 24ABPM SBP ≥ 130 mmHg. 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 ± 9 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 vs. 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 accounted for 69% of the variance in MH. Light intensity exercise brachial SBP was independently associated with MH, and if ≥175 mmHg, identified MH with 75% sensitivity and 67% specificity (P < 0.005). MH can be identified in untreated individuals from low intensity exercise brachial SBP but not resting PWA. Further research on the diagnostic value of BP during early phases of exercise stress testing is needed.

081

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 South Africans, but their IGF-1 concentration is unknown. We aimed to compare serum IGF-1 concentrations of African and Caucasian people; to investigate their age-related IGF-1 decline and to determine whether IGF-1 could account, at least in part, for the high prevalence of noncommunicable diseases in black Africans. This cross-sectional study involved 211 African and 316 Caucasian men and women (aged 20–70 years). 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, PWV 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.

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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 antihypertensive 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 ± 8 years, 58% male), were randomised to 3 months spironolactone (25 mg daily; n = 57) or placebo (n = 55). An EEBP was defined as brachial BP ≥ 190/105 mmHg (men) or ≥ 210/105 mmHg (men) during maximal exercise. Arterial stiffness was recorded by arterial wave velocity (PWV). Brachial BP was estimated by sphygmomanometer 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), VO2 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 VO2 max (r = –0.29; 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 < 0.0007) but did not change aortic PWV, VO2 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/ ms2) were significantly reduced during low-stress exercise (P < 0.05 for both), whereas low-stress central SBP was unchanged by spironolactone (–3.8 ± 16.3 versus 1.0 ± 13.1 mgHg; P = 0.14). We conclude that maximal exercise BP, as well as submaximal central systolic loading during light activity, are improved by spironolactone in patients with EEBP, but these changes cannot be attributed to reduced central artery stiffness.

083

IMPROVED QUALITY OF LIFE FOLLOWING UNILATERAL LAPAROSCOPIC ADELRENYECTOMY IN PATIENTS WITH UNILATERAL PRIMARY ALDOSTERONISM
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For patients with unilateral primary aldosteronism (PAL), unilateral laparoscopic adrenalectomy (AD) corrects hypertension 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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QOL, with significant QOL improvement observed as early as 3 months post-ADX and persisting...

[44x619]/**P*/0.001) and renin concentration (/**P*/0.01) rose, with 86% of these patients cured of hypertension (BP...

Mean SBP and DBP improved significantly (**P*/0.001), systolic blood pressure (**r*/0.32, **P*/0.001) and fitness (**r*/0.03) and positively with change in...

**EXERCISE AUGMENTS WEIGHT LOSS INDUCED IMPROVEMENT IN RENAL FUNCTION IN OBESE METABOLIC SYNDROME SUBJECTS**

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The metabolic syndrome (MetS) is independently associated with an increased risk for incident chronic kidney disease. The objectives of this study were to examine (1) the effects of lifestyle intervention on renal function in this clinical setting and (2) correlates of improved renal function. Thirty eight MetS subjects (23 M, 15 F; mean age 55±1.1 yrs; BMI 32.7±0.6 kg/m²) who fulfilled NCEP ATP III criteria were randomized to 12-weeks dietary weight loss intervention on renal function in this clinical setting and (2) the correlates of improvement in renal function.

**EFFECT OF HYDROGEN SULPHIDE IN THE BRAIN ON CARDIOVASCULAR REGULATION**

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Hydrogen sulphide has long been known for its smell and toxicity. In the last decade, however, hydrogen sulphide has been found to have several physiological effects including vasodilatory and neuromodulatory roles. In the cardiovascular system, hydrogen sulphide has been shown to have direct vasorelaxing and cardiovascular regulatory effects. More recently it has been suggested that hydrogen sulphide affects the brain to reduce blood pressure. In the present study we have investigated the effects of microinjecting hydrogen sulphone donors and the effects of inhibiting endogenous hydrogen sulphone production on blood pressure (BP), heart rate (HR) and lumbar sympathetic nerve activity (LSNA) in anaesthetised Wistar-Kyoto rats. We have demonstrated that intravenous infusion increases blood pressure, induces proteinuria and increases soluble FMS-like tyrosine kinase 1 (sFlt-1) plasma concentrations in pregnant baboons.

**TUMOR NECROSIS FACTOR ALPHA INDUCES PLACENTAL ANTIANGIOGENIC FACTORS IN PREGNANT BABAONS (PAPIO HAMADRYAS)**

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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 the blood pressure (BP) profile of baboons; including day time values, night time values and the MSBP to determine if the baboon has a similar BP profile to the human. Pregnant (n=3) and non-pregnant (n=5) female baboons had a telemedical (Palm, Australia) telemetry unit fitted to monitor 24-hour intra-arterial blood pressure measurement. The animals were kept on a day-night cycle using artificial timed light from 6am - 6pm daily. The MSBP was defined as the BP 2 hours after rising (8 am) minus the average BP overnight (6pm–6am). Data from 2 mornings and nights was averaged, and t-tests were performed for statistical analysis using PASW Statistics 17.0 software (SPSS Inc., USA). There were no significant differences between the groups in age or weight of the animals. In the non-pregnant animals, mean overnight BP was 127±2±2 mmHg systolic and 83±2±2 mmHg diastolic and morning BP was 139±4±4 mmHg systolic and 90±1±1 mmHg diastolic. The pregnant animals was 112±3±3 mmHg systolic (**P*/0.004) and 76±3±3 mmHg diastolic (**P*/0.010) and in the morning (122±3±3 mmHg systolic (**P*/0.028) and 79±3±3 mmHg diastolic (**P*/0.044). The mean MSBP was 12±3±2 mmHg systolic and 7±2±2 mmHg diastolic for the non-pregnant baboons, and 10±2±2 mmHg systolic and 3±2±2 mmHg diastolic for the pregnant baboons (not significantly different, **P*/0.699 and **P*/0.308). These results suggest that baboon blood pressure is similar to humans in terms of the MSBP, with a rise in systolic BP of 10–15 mmHg in the morning. Pregnancy does not appear to affect the presence or magnitude of the MSBP. Also similarly to humans, the blood pressure of pregnant females is significantly lower than that of non-pregnant females.

**084 MORNING SURGE IN BLOOD PRESSURE IN PREGNANT AND NON-PREGNANT TELEMETRED BABOONS (PAPIO HAMADRYAS)**

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Human blood pressure follows a diurnal pattern, peaking by mid-morning and falling progressively throughout the day to reach its nadir at 3 am during sleep. The incidence of cardiovascular events such as stroke, transient ischaemic attack, sudden cardiac death and myocardial infarction peaks in the morning, following a relative decline in these events during the night. It is thought that the morning surge in blood pressure in pregnant women may contribute part of the explanation for the increased risk of cardiovascular events in pregnancy.

The aim of this study was to investigate the blood pressure (BP) profile of baboons; including day time values, night time values and the MSBP to determine if the baboon has a similar BP profile to the human. Pregnant (n=3) and non-pregnant (n=5) female baboons had a telemedical (Palm, Australia) telemetry unit fitted to monitor 24-hour intra-arterial blood pressure measurement. The animals were kept on a day-night cycle using artificial timed light from 6am - 6pm daily. The MSBP was defined as the BP 2 hours after rising (8 am) minus the average BP overnight (6pm–6am). Data from 2 mornings and nights was averaged, and t-tests were performed for statistical analysis using PASW Statistics 17.0 software (SPSS Inc., USA). There were no significant differences between the groups in age or weight of the animals. In the non-pregnant animals, mean overnight BP was 127±2±2 mmHg systolic and 83±2±2 mmHg diastolic and morning BP was 139±4±4 mmHg systolic and 90±1±1 mmHg diastolic. The pregnant animals was 112±3±3 mmHg systolic (**P*/0.004) and 76±3±3 mmHg diastolic (**P*/0.010) and in the morning (122±3±3 mmHg systolic (**P*/0.028) and 79±3±3 mmHg diastolic (**P*/0.044). The mean MSBP was 12±3±2 mmHg systolic and 7±2±2 mmHg diastolic for the non-pregnant baboons, and 10±2±2 mmHg systolic and 3±2±2 mmHg diastolic for the pregnant baboons (not significantly different, **P*/0.699 and **P*/0.308). These results suggest that baboon blood pressure is similar to humans in terms of the MSBP, with a rise in systolic BP of 10–15 mmHg in the morning. Pregnancy does not appear to affect the presence or magnitude of the MSBP. Also similarly to humans, the blood pressure of pregnant females is significantly lower than that of non-pregnant females.

**085 TUMOR NECROSIS FACTOR ALPHA INDUCES PLACENTAL ANTIANGIOGENIC FACTORS IN PREGNANT BABAONS (PAPIO HAMADRYAS)**

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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 there was a correlation between increased sFlt-1 plasma concentrations and the development of hypertension in the pregnant baboon. 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 there was a correlation between increased sFlt-1 plasma concentrations and the development of hypertension in the pregnant baboon.
THE RECRUITMENT OF BLOOD-BORNE QDOT-LABELLED CELLS INTO ATHEROPLAQUE

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We have come full circle is determining whether bone marrow or blood borne 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/day GCSF and on the fifth day, CD45- blood 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 106 QDot-labeled autologous cells were injected weekly during this time. Viability of cells in vivo was confirmed the day after using flow cytometry. Labelled blood cells, the ascending aorta and left main coronary artery (LMCA) were then studied by confocal microscopy. QDot-positive ‘neo’ endothelial cells were visible in both the ascending aorta and the LMCA, which appeared to be concentrated on the shoulder of the plaque in the LMCA. However, QDot-positive neo-internal cells and medial cells were only found in the LMCA, 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-internal 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 VELOCITY 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-anaesthetized rabbits and the ascending biaxial and left main coronary arteries were studied at baseline and after the resting HR was lowered with multiple doses of a bradycardic agent (zatebradine, 1mg/kg, i.v.). Effects of HR on aPWV at different mean arterial pressures (MAPs) (30, 40 and 450 mm Hg) were applied to compare means of aPWV at 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 MAP respectively (30-160 mmHg). We conclude from this study that the haemodynamic effects of the ACE inhibitor, perindopril are at least partially mediated by activation of both AT1 and AT2 receptors, possibly involving stimulation by the angiotensin peptide fragment, Ang (1-7), which acts as a counter-regulatory pathway against the pro-atherogenic ACE/Ang II/AT₁R axis.

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 antihypertensive 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 T2DM is common in T2DM 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% were female, mean age at diabetes diagnosis was 49±13 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: Group A subjects were normotensive (BP <130/80) and on no AHT (n=75, 6.2%); subjects in Group B were at BP goal of <130/80 and on AHT (n=195, 16.1%); Group C subjects had not achieved BP goal of <130/80 and were on ≥3 AHT (n=705, 58.1%); and Group D subjects were classified as resistant HTN i.e. not at BP goal and were on ≥3 AHT (n=238, 19.7%). Group B 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 B had abnormal LV hypertrophy 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.
Aortic stiffening, 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 (whilst seated and standing), at home (7-day average) and by 24 hour ambulatory monitoring (24ABPM). Clinical central BP and aortic pulse wave velocity (PWA) (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 PWA (tertile 1: 19 ± 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 PWA (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 domain. 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.

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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 levocarmilol (LK)-induced vasodilation, was significantly attenuated (P<0.05 ANOVA) in diabetic rats (EC50 87.7±1.5 % maximum relaxation; EC50 118 mM, 95% CI 77–178 mM; n=18) compared to control rats (EC50 96.0±0.9 %, EC50 53 mM, 41–88 mM, n=17). Inhibition of the nitric oxide (NO) pathway using l-NAME (100 μM) and charybdotoxin (0.1 μM), respectively, inhibited the EDHF pathway with apamin (1 μM) and iberiotoxin (0.1 μM), respectively. Inhibition of the EDHF pathway with apamin (1 μM) together with the intermediate- and large-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 ibotrexato (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.

FENOFRIBATE 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 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 NO3 concentrations as a marker of NO production. Relative heart and LV volumes were significantly increased in ACTH- and dexamethasone-treated rats but had no effect on thymus weight, plasma NO3 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 MICROSCOPE

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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 LPD hearts only (P<0.009 and P<0.04, 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.
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