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

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The C1 neurons of the rostral ventrolateral medulla (rVLM) innervate sympathetic preganglionic neurons in the spinal cord that convey sympathetic vasomotor tone, and the catecholaminergic neurons of the locus coeruleus (LC) and A5 region. In this study, we investigated the effects of C1 neuron stimulation on sympathetic vasomotor discharge and the activity of LC and A5 neurons. We used a lentivirus that expresses a fusion protein between the light-sensitive Channelrhodopsin 2 (ChR2) and the fluorescent reporter mCherry under the control of the PRSx8 artificial promoter (a Phox2-responsive element) to drive ChR2-mCherry expression selectively into Phox2b-positive neurons (89 ± 1% N = 12). Virus microinjections into the LC region produce ChR2-mCherry expression in C1 neurons and non-C1 Phox2b-positive neurons in the retrotrapezoid nucleus (1:1 ratio of C1: non-C1 neurons, 70% of all C1 neurons). Pulsed photostimulation of the rVLM with 473 nm laser light (LL) in vivo (10 ms pulses, 20 Hz, 30% increment on arterial pressure (AP) by 13.8±2.2 mmHg and splanchic sympathetic nerve discharge (sSND) by 60.3% (N = 9). Following chemical blockade of the sympathetic baroreflex, photostimulation produced a significantly greater increase in arterial pressure (P < 0.01; N = 6) and SND (P < 0.05; N = 6). Single LL pulses produced a massive evoked burst of SND (peak; 1433 ± 755/11005 mmHg sSND) with latency 28 ± 1 ms; N = 9) followed by a long-lasting reduction of SND (18.9 ± 5.5% relative to baseline). Twin-pulse stimulation revealed a significant reduction in the amplitude of the second pulse if delayed less than 2 s after the initial pulse. Low frequency photostimulation of the LC region produced a temporarily precise activation of both LC (16±22; N = 4) and A5 (3/8; N = 2) neurons. Intracerebroventricular administration of kynurenic acid blocked C1 evoked excitation of LC neurons (12/12). These results confirm that the C1 neurons are sympathetic-vascular 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 factor for cerebro- and cardio-vascular diseases. Although elevated blood pressure (BP) is usually controllable by therapeutic means, diet based strategies to lower BP are becoming increasingly popular, as they may act as a safer and more cost-effective alternative when compared to conventional drug therapy. Recent efforts have identified potential therapeutic roles in human health for specific dietary components (bioactives). For example, several bioactive peptides which inhibit ACE and/or antagonise the AT1 receptor have been isolated, and products consequently formulated. In addition, 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 125mg/kg) and 6hr (21%; 250 mg/kg) time points. In comparison, Enalapril (4mg/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, EC25) 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 volunteer studies, the Asklepios group, and correspond to the low amplification of the pulse between carotid and brachial sites. The current method of calibrating the radial waveform to brachial cuff pressure is more appropriate than using brachial tonometry. Invasive studies of simultaneously recorded brachial and radial waveform will be necessary to confirm or refute this view.

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 aortic pressure to aortic pressure waveforms and aortic pressure to aortic flow waveforms. Such a process can be used (when relevant aortic diameter is known) to estimate of stroke volume and cardiac output, and was studied in this report. Flow velocity waveforms in the left ventricular outflow tract were measured by Doppler from the apical window 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 wave was synthesised from the first 5 harmonics with flow >1 cm/s. The formula was: Flow (cm/s)=Pressure (dyne cm^-2)− Impedance (dyne.s.cm^-3).

Flow waveforms at different ages were realistic, with peak flow corresponding to the first systemic 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 56 ml (calculated) c.f. 81 ml (measured by Doppler), and cardiac output averaging 3.7 L/min (calculated) c.f. 5.1 L/min (measured by Doppler). The double TF method (pressure -- pressure then pressure -- flow), is physiologically appropriate under control conditions, and appears to provide realistic flow from the heart, calculated from pressure waveforms and modelled impedance. Systematically lower calculated values may be true, and due to measurement of Doppler flow velocity from the outer envelope of the wave.
MEASUREMENT OF MEAN ARM PRESSURE FOR CALCULATION OF CENTRAL AORTIC SYSTOLIC AND PULSE PRESSURE

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In 1992, Kelly and Frikhail [JACC 1992;20:652] proposed that the carotid pressure waveform, measured non-invasively by applanation tonometry, could be calibrated from upper arm mean and diastolic pressure, with the assumption that these values were identical in central and peripheral vessels. This has been widely used since but the practice has been questioned when mean pressure (MP) is calculated from diastolic pressure plus a fixed proportion (Form Factor (FF) of 33% of pressure pulse (PP)) [Hypertension 2009;54:414]. This study was undertaken to establish in a clinical database, the value and range of FF in tonometric radial artery waveforms, and in aortic waveforms calculated from a generalised aortic-radial transfer function, and its dependence on age, MP, heart rate (HR), ejection duration (ED), PP amplification (PPA), brachial PP and aortic augmentation index (AxA). The database [JACC 2008;2:28] comprised 8212 observations in 1500.

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

<table>
<thead>
<tr>
<th>Mean</th>
<th>SD</th>
<th>R² for correlation</th>
<th>Regression</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radial FF (%)</td>
<td>33.6</td>
<td>4.4</td>
<td>0.004</td>
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<tr>
<td>Age (y)</td>
<td>69.4</td>
<td>13.2</td>
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<tr>
<td>HR (bpm)</td>
<td>66.6</td>
<td>12.7</td>
<td>0.009</td>
<td>&lt;0.01</td>
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<tr>
<td>ED (msec)</td>
<td>318.1</td>
<td>32.4</td>
<td>0.077</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MP (mmHg)</td>
<td>95.3</td>
<td>13.3</td>
<td>0.263</td>
<td>0.001</td>
</tr>
<tr>
<td>PPA (%)</td>
<td>21.7</td>
<td>9.2</td>
<td>0.272</td>
<td>&lt;0.001</td>
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<tr>
<td>AxA (%)</td>
<td>27.1</td>
<td>11.9</td>
<td>0.334</td>
<td>0.21</td>
</tr>
<tr>
<td>PP (mmHg)</td>
<td>66.6</td>
<td>17.9</td>
<td>0.000</td>
<td>0.03</td>
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Wide variation in radial artery FF results in substantially different values of MP being calculated if a set formula such as diastolic + 33% of PP is used. The value of radial artery tonometry is that it provides an arithmetic averaged MP over the cardiac cycle, so that no MP is fixed. 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 EXPLOSE 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 (n-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 a diet deficient in vitamins and minerals except for Se. The diets were either sufficient (Se-rich diet) or deficient (n-3 fatty acid deficient diet). At 7 weeks of age, male Sprague-Dawley rats (N=40) were divided into four groups and placed on semi-synthetic diets that contained identical amounts of 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 n-3 fatty acid deficiency.

<table>
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AN INCREASED SALT INTAKE IS ASSOCIATED WITH AN INCREASED HYPERTENSIVE RESPONSE TO STRESS IN PATIENTS WITH ESSENTIAL HYPERTENSION

A. Deguzman1, C. Aragon2,3,4, J. Ochoa5,6, A. Lopez1,2,3,4, J. Ochoa5,6

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

EFFECTS OF BLOOD PRESSURE LEVELS AT PRESENTATION AND ACHIEVED IN THE FIRST 24 HOURS ON HAEMATOMA GROWTH IN ACUTE 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 by experienced radiologists. The average 12-hour change of BP levels on absolute and percentage change in haematoma volumes were assessed by an analysis of covariance (ANCOVA) with age, sex, log of baseline haematoma volume, haematoma location, time from CT to ICH onset, and randomised treatment, as covariates. Overall, 346 patients with 2 CT scans were available for analyses. Absolute growth in haematoma volume increased with higher baseline BP levels: 2.1 ml (95% CI: 0.6 – 4.6 ml), 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: 2.5 –7.5 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 provide 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 results in NOS, 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 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 measured by immunoblotting and 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<0.001). 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 x̅±0.6; P<0.01 and 1.6 x̅±0.1; P<0.01, respectively), whereas NOX4 expression remained unchanged. In tissue sections NOX1 showed strong positive staining in the intima of SHR, where 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±1.1% versus WKY: 67.9±2.7%, P<0.05). The NADPH oxidase inhibitors VAS2870 (10 μmol/L) and apocynin (100 μmol/L) improved the relaxation in aortae of both WKY rats (79.4±2.2% and 80.2±2.6%, respectively) and even more pronounced in SHR (80.6±5.6% and 77.8±4.9%, respectively) resulting in similar relaxation of SHR and WKY aortae. In conclusion, ROS formation is increased in aged SHR and hence represent a novel target for blood pressure reduction via inhibition of eNOS.

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 weeks fat feeding in rabbits results in a greater vescular fat pad 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), tracylglyceride (TAG), ceramides and cholester (CE) were obtained after analysis by liquid chromatography mass spectrometry. We measured 6 species of ceramide, 22 of DAG, 43 of TAG and 29 CE's. HFD rabbits exhibited greater plasma concentrations of total measured ceramides (P<0.05) and TAGs (P<0.05). Five of the six ceramide species were significantly increased in HFD compared with ND rats. 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<0.05 and TAG (P<0.01) did not differ between dietary groups. Only one DAG (16,0, 20,4, P<0.04), and one TAG (18, 18, 20,4, P=0.02) was increased 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 sequesteration 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.

HIGH-FAT FEEDING ALTERS THE CARDIOVASCULAR ROLE OF THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS

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Increased sympathetic nerve activity is associated with obese-related hypertension, but the underlying central neural mechanisms are not clear. We examined the role of the hypothalamic paraventricular nucleus in the regulation of sympathetic nerve activity in rats fed either a normal chow (controls) or a high fat diet (56% fat) over 12 weeks. The effects on blood pressure, heart rate and lumbar sympathetic nerve activity induced by microinjection of the GABA(A) receptor agonist, muscimol, or the antagonist, bicuculline, were monitored in anesthetized rats. Compared with chow-fed rats, rats fed a high fat diet were significantly different in body weight but had a significant 80% increase in epididymal fat mass, significantly elevated fasting blood glucose and a significantly impaired glucose tolerance. Resting blood pressure and heart rate were not significantly different in rats fed a high fat diet or normal chow diet. Muscimol microinjected into the paraventricular nucleus increased blood pressure and lumbar sympathetic nerve activity but the responses were similar in high fat and control rats. In conclusion, high fat feeding may alter the influence of the paraventricular nucleus on cardiovascular variables prior to the development of hypertension and obesity.

CYTOCHROME P450 ENZYMOGENE 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 by activating large conductance Ca2+−activated K+ channels and have been proposed as endothelin derived hyperpolarizing factors (EDHF). In addition, they have proinflamatory activity, anti-inflammatory actions and are inhibitors of platelet aggregation and smooth muscle cell proliferation. The physiological activity of their metabolites, dihydroxyeicosatrienoic acids (DHEAs), are dependent on their metabolism by cytochrome P450 (CYP450) oxygenases. In a previous study, we have showed that metabolism of arachidonic acid by CYP450 ω-hydroxylation 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 diet-induced insulin resistant rabbits, 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=16) matched for age and gender. EETs and DHEAs 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±1.5y (controls) with blood pressure and BMI of 135/87±2.7/1.8mmHg and 34.3±3.1kg/m2 respectively, (MetS); and 112/69±2.1/1.6mmHg and 24.2±2.1kg/m2, controls. Plasma EETs were significantly increased in the MetS (7.7±0.9ng/ml compared with controls (6.2±0.3ng/ml, P=0.007). Plasma DHEAs were not different between the groups 11.6±0.95ng/ml (MetS) compared with 11.5±1.06ng/ml (controls). In contrast to plasma, platelet EETs were significantly reduced in the MetS (1.4±0.1ng/106cells) compared with controls (2.1±0.31ng/106cells, P<0.04). Platelet DHETs were not different between the groups (3.9±0.3ng/106cells MetS vs 4.1±0.3ng/106cells controls). The ratio of DHETs to EETs was calculated as an index of eNOS activity in plasma and platelets but was not significantly different between the groups. The increase in plasma EETs in the MetS may be a homeostatic response to elevated blood pressure or increased circulating vasconstrictors that have been linked to insulin resistance in these subjects. The reduced platelet EETs levels may be of relevance to increased platelet reactivity and aspirin resistance that has been described in subjects with 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, hypothalamic 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 angiotensin II receptor 1a (AT1a) expression was up-regulated 3.27 fold (n=6; P<0.001). By 3-months AT1a was a non longer over-expressed. Overall, these findings demonstrate the involvement of the RAS in hypertension related to ω-3 fatty acid deficiency and indicate that early programming may be involved. Furthermore, reducing RAS activity can effectively treat the blood pressure increase caused by ω-3 fatty acid deficiency.
CVD IN INDIGENOUS AUSTRALIANS: OPPORTUNITIES FOR IMPROVING OUTCOMES ACROSS THE CONTINuum OF CARE

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Whilst recent political and health system reform has focused on ‘closing the gap’ in health status for Indigenous Australians, less attention has been afforded to outlining the specific activities which are most likely to reduce this gap.

Amongst a long list of health issues driving the disparity experienced by Indigenous Australians, Cardiovascular Disease (CVD) remains the primary target. They are the principal cause of death and of excess death among Indigenous people in Australia, and account for almost one-third of the life expectancy gap. More particular is the significant disparity at younger ages, with both Aboriginal men and women up to ten times more likely to die from CVD between the ages of 25–54 years. In terms of explaining these gaps, most attention has focused on the higher burden of traditional risk factors experienced by Indigenous people. Far less attention has focused on the quality and outcomes of health system performance in explaining these differentials. What is known is that amongst a long list of health issues driving the disparity experienced by Indigenous Australians, less attention has been afforded to outlining the specific activities which are most likely to reduce this gap. Peptides, high and low molecular weight kininogens, plasma kallikrein or kallistatin.

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

INCREASED TISSUE KALLIKREIN EXPRESSION IN HUMAN TYPE 2 DIABETES

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

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associated with night-time active periods in the Schiødt (PHV/LJ) hypertensive mice leading to hypotension and an exaggerated circadian day-night difference in BP.

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

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The lateral parabrachial nucleus (LPBN) is increasingly viewed as a critical integrative site for the transfer of visceral cardiovascular information from the brainstem to a number of regions in the hypothalamus. The goals of this study were to examine the topography and relationship between BP sensitive neurons located in LPB subnuclei and their projections to the median preoptic nucleus (MnPo) and central nucleus of the amygdala (CeAm). Retrogradely transported neuronal tracer was injected into either the MnPo (n=8) or the CeAm (n=8) and rats underwent a 1-week recovery period. C-Fos immunohistochemistry was used to detect neurons in the LPBN activated in response to iv infusion of phenylephrine hydrochloride (10 μg/kg/min; n=8) or sc injection of diazoxide (45 mg/kg; n=8) in experimental groups; or iv infusion or sc injection of isotonic saline (45 mg/kg; n=8) in control groups. Neuronal activation in rats infused with iv phenylephrine was greater in the central LPB, central part of the external, and superior lateral subnuclei (P<0.001) compared with vehicle infused controls. The findings demonstrate that efferent projections arising from neurons in the central (43%), dorsal (31%) and dorsal part of the external (69%) lateral subnuclei innervating the MnPo also respond to an increase in BP. Additionally, 63% of neurons located in the central part of the external lateral FBN that innervated the MnPo were sensitive to a rise in BP. Although there was neuronal activation in rats with reduced arterial pressure induced by sc injections of diazoxide was greater in the central LPB and central part of the external LPB subnuclei compared with saline infused controls, these cells were not retrogradely labelled from either the MnPo or the CeAm. This is the first demonstration of direct neural projections arising from pressor responsive neurons in discrete LPB subnuclei innervating neurons in the MnPo and CeAm. Although, there was no evidence for ascending projections from neurons activated by hypotension terminating in either the MnPo or CeAm, this study does provide topographic evidence for separate populations of neurons in the LPBN responding to elevated and reduced BP.

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

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

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Hydrogen sulphide is a gaseous mediator endogenously generated in the heart and blood vessels. It has been shown to cause vasorelaxation and plays a role in cardioprotection from 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 six normal healthy volunteers and incubated with 10 μM hydrogen sulphide or pH matched vehicle control for 20 seconds prior to initiation of platelet activation and aggregation with chemical agonists. While samples treated with hydrogen sulphide exhibited normal shape change and initiated primary aggregation, secondary platelet aggregation was inhibited by hydrogen sulphide versus vehicle control in response to 0.5 μM arachidonic acid (5.6% vs 81.4%, P<0.001). 2 μM collagen (28% vs 82%, P<0.001), 0.5 μM of thromboxane mimetic U46619 (38.4% vs 67.7%, P<0.001) and 7 μM adrenaline (19.6% vs 76.5%, P<0.001). 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 secretion of important signaling molecules such as ATP, ADP, adrenaline, ionin 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 the rich thrombin signal only engaging 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.
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 CHCT1/2 gene polymorphisms with hypertension, 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 maternal methylenetetrahydrofolate reductase (MTHFR) genotype was associated with healthy pregnancies (P < 0.03, respect). Neonatal NAT1*10 T allele corrected for age and BMI was associated with maternal mean arterial pressure and sBP (P = 0.011, P = 0.001). MTR and MTHFR to healthy pregnancies (P < 0.008 & P < 0.003, respectively). Basal and stimulated heart function was not altered, although basal 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 vasoconstriction may be factors which contribute to the increased susceptibility to ischemia/reperfusion injury.

ASSOCIATION WITH HYPERTENSION IN PREGNANCY

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Vitamin D is a fat-soluble vitamin which is essential in bone metabolism, cell growth, differentiation and regulation of membrane fluidity. The aim of the present study was to investigate the effect of vitamin D deficiency on cardiac function, using echocardiography, in 14 week old adult rats. Four week old Sprague-Dawley female rats were fed either a vitamin D-deplete or vitamin D replete (control) diet for 6 weeks prior to pregnancy, during pregnancy and throughout lactation. At weaning the offspring remained on their respective diets until adulthood. At 14 weeks of age non-invasive trans-thoracic echocardiography was performed in female offspring (n = 9) and male offspring (n = 11). The effect of vitamin D deficiency was studied using echocardiography. By comparison of 14 week old vitamin D deficient and vitamin D supplemented animals, the effect of vitamin D status on the heart was assessed. The left ventricular weight to body weight ratio was significantly increased in vitamin D deficient offspring (P < 0.001). Vitamin D deficiency was accompanied by a significant increase in diastolic volume (P = 0.0002) but no difference in systolic volume. In addition, stroke volume and cardiac output in the vitamin D deficient offspring were significantly reduced (P = 0.001). Fractional shortening and ejection fraction were unaltered in both control and vitamin D deficient female offspring. In conclusion, long term vitamin D deficiency in female rats leads to left ventricular hypertrophy and impaired cardiac function. The findings are in agreement with clinical data whereby vitamin D deficiency is linked to heart disease.

EFFECT OF VITAMIN D DEFICIENCY ON CARDIAC FUNCTION AND SUSCEPTIBILITY TO ISCHEMIA/REPERFUSION INJURY IN THE ADULT RAT HEART

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Exposure to vitamin D deficiency in utero and early life leads to delayed maturation and subsequent enhanced growth (proliferation and hypertrophy) of cardiomyocytes in the left ventricle. The implications of these changes on cardiac function later in life are unknown. The aim of the present study was to investigate the effect of vitamin D deficiency in adult rats on cardiac function and the susceptibility to ischemia/reperfusion injury. Four week old Sprague-Dawley female rats were fed either a vitamin D-deplete or vitamin D replete (control) diet for 6 weeks prior to pregnancy, during pregnancy and throughout lactation. Offspring remained on their respective diets until adulthood. Hearts of 16 week old male and female offspring (n = 8/group) were harvested on a Langendorff apparatus. Basal heart rate (HR), coronary flow, rate of contraction (+dp/dt) and relaxation (-dp/dt) and response to isoprenaline were recorded. The hearts were then subjected to 20 minutes ischemia and 1 hour 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 = 0.008 & P = 0.003, 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 vasoconstriction may be factors which contribute to the increased susceptibility to ischemia/reperfusion injury.

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NEUROPEPTIDE CODING OF ADRENALLY PROJECTING SYMPATHETIC PREGANGLIONIC NEURONS

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Different physiological stimuli selectively evoke release of noradrenaline (NA) or adrenaline (Ad) from the adrenal medulla. Therefore NA and Ad chromaffin cells are innervated by different populations of sympathetic preganglionic neurons (SPN). How these populations differ functionally is not been consisitent although 21 genes were associated with blood pressure in female and peripheral nervous systems, in adrenal glands, adipose tissue, and peripheral blood mononuclear cells. RT-PCR was used to validate these observations and enabled the identification of several splice variants of the reninase transcript. These splice variants appear to be tissue-specific and point to a role of reninase function in the structural and functional characteristics of renal cell to facilitate our ability to diagnose and treat disorders involving an imbalance in the levels of mononorme neurotransmitters. We are currently pursuing investigations into the regulation of reninase gene expression using a variety of cell culture and animal models.

INCREASED NO-DEPENDENT DILATION OF RAT ARTERIOLES IN DIET-INDUCED OBESITY

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Obesity is an established risk factor for hypertension and associated cardiovascular disease and impairs blood vessel function. The large-conductance Ca2+–activated potassium channel (BKCa) has a major regulatory control role in the cardiovascular system and is involved in the regulation of vascular tone and pressure. However, the specific role of BKCa function in rat skeletal muscle arteries in diet-induced obesity is not yet defined. The aim of this study was to examine the effect of diet-induced obesity on endothelium- and BKCa-dependent dilation of rat skeletal muscle arteries. Male Sprague-Dawley rats (7–8 weeks) were fed a cafeteria-style high fat or control diet for 16 weeks. Following anesthesia with sodium thioneptrone (100 mg/kg i.p.), studies were performed in isolated, pressurized (70 mmHg) first-order arterioles from the cremaster muscle. Control rats weighed 570±7 g compared with obese rats 768±13 g (n=37–35 of each, P<0.05). Electronic microscopy of cremaster muscle arterioles from both media thickness and to lumen ratio significantly increased in diet-induced obese rats (n=10–10 of each). Despite this, diet-induced obesity had no effect on acetylcholine (ACH; 0.001–3 μM)-induced dilation of arterioles. The NOS and guanylate cyclase (GC) inhibitors L-NAME (100 μM) and ODQ (10 μM) did not alter myogenic tone in arteries from control rats, however, in arterioles from obese rats diameter was significantly reduced (obese baseline 45.5±1.2%, L-NAME/ODQ 38.1±1.6%, n=21, P<0.05). ACh-induced dilation of arterioles from control rats was completely abolished by a combination of the BKCa blockers 5-hydroxytriptamine-34 (1 μM) and amiloride and barbiton (BTX, 0.1 μM) respectively, with no apparent role for NO as assessed by the lack of effect of L-NAME/ODQ (n=10–15 of each). In arterioles from obese rats however, BTX had no effect on responses to ACh while the NO/GC inhibitors partially inhibited ACh-induced dilation (maximum, 3 μM ACh, 97.2±2.4% v maximum L-NAME/ODQ 82.6±2.7%, n=12–15 of each, P<0.05). Western blotting showed decreased expression of the BKβ1, β3-subunit in arterioles from obese rats. Expression of eNOS was not altered by obesity, however there was an accumulation of complexed forms of caveolin-1 and -2 at cremaster arteriole membranes. In summary, diet-induced obesity resulted in an alteration in the relative contribution of NO and BKCa to ACh-mediated endothelium-dependent dilation of rat cremaster muscle arterioles. BKβ1–dependent dilation was abolished in obesity while an NO-component was established.

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

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Childhood blood pressure is predictive of adult hypertension. Genes of the hypothalamic pituitary axis may affect blood pressure control. In adult studies, association studies have shown that the HNR3C2 variant of the insulin-like growth factor, 2 receptor is associated with diabetes or obesity and may be related to BP and hyper-tension. The aim of the study was to identify genes which are related to longitudinal blood pressure in childhood. The Raine Pregnancy Cohort (n=2868) has recorded oesociliometric resting blood pressure on children at age 1, 2, 3, 5, 8, 10 and 14 years. Linear mixed effects was used to identify trajectory for each individual. Forty-four genes related to hypertension and 42 SNPs genes of 15 genes were associated with blood pressure in males (all P<0.05). The single nucleotide polymorphisms (SNPs) from the list of genes were analysed by multivariate linear modelling for association with resting blood pressure on children at age 1, 2, 3, 5, 8, 10 and 14 years. Linear mixed effects was used to identify trajectories and intercepts for each individual. Forty-four genes related to the hypothalamic pituitary axis were selected and tagged to ensure complete coverage of the gene taking into account linkage disequilibrium blocks. The single nucleotide polymorphisms (SNPs) from the list of genes were analysed by multivariate linear modelling for association with systolic blood pressure intercept and trajectory separately for boys and girls. Of the 44 HPA axis genes, 20 genes (45%) were associated with systolic blood pressure in females and 42 SNPs genes of 15 genes were associated with blood pressure in males (all P<0.05). The following SNP’s were associated with blood pressure trajectories or intercepts: 15 SNPs of nuclear receptor subfamily 3 (NR3C2), 12 SNPs of insulin growth factor 1 receptor (IGF1R), 6 SNPs of insulin receptor growth factor 2 receptor (IGF2R), 9 SNPs of proopiomelanocortin (POMC) and 5 SNPs of leptin and 5 SNPs of leptin receptor. Increased BP trajectories in adolescence was associated with SNPs in 10 genes in males, 13 genes in females and 6 genes common to both sexes (FGF2r, IGF2r, CFR, INS, INS2, INS12Q1, INSR, MS1, INSR, PCSK1, INSR). Conversely, decreased BP in adolescence was associated with SNPs in 11 genes in males, 10 genes in females and 3 genes common to both sexes (GHR, NR3C2, PCSK1). In conclusion, this study suggests that the
genes of the hypothalamic axis, in particular NR3C2, IGF1R, IGF2R, PSCK2, 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 urine excretion. A cross-sectional study was conducted using participants enrolled in the Melbourne Collaborative Cohort study. Daily intakes of sodium and potassium were measured from 24 hr urine samples provided by participants (2007/08). BP was assessed under standard conditions in a subgroup of this population. The mean age of participants (men n = 376, women n = 408) was 64.0 (6.3) (SD) years. For men and women respectively, the mean urinary sodium was 178.4 (68.6) and 133.7 (51) mmol/day (approximately 10 g and 8 g salt/day), mean urinary potassium was 88.1 (30.7) and 71.0 (23.9) mmol/day and the sodium to potassium (Na:K) ratio was 2.16 (0.88) and 1.93 (0.74) g/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 (0.69) 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 (β = 0.013, P = 0.013), but not for diastolic BP. In conclusion, in 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 (sNSA) 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 performed 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 (3 kg/kg) adult male Sprague-Dawley rats (n = 12) were vagotomised, ventilated, and paralysed. MAP, HR (derived from ECG) and end tidal CO2 were monitored, and SNSA was recorded. A catheter was inserted into the IT space and advanced to the level of T6. In n = 12 animals, an initial control injection of 10 µl of 10 mM PBS was given. In n = 6 of the animals, 10 µl of 1 mM PACAP-38 (a specific PAC1 receptor antagonist) was injected. Responses were recorded for 30 mins. In the remaining animals (n = 6), pretreatment with PACAP(6-38) was given 15 mins before 10 µl of 1 mM PACAP-38 (a non-specific agonist) was administered and responses were recorded for 60 mins. PACAP6-38 alone caused no significant change in MAP (7 ± 5 mmHg), HR (5 ± 2 bpm) or sNSA (10 ± 7 %). Following pretreatment with PACAP6-38, PACAP-38 caused significant changes in MAP (-20.7 ± 7 mmHg), HR (17 ± 4 bpm) and sNSA (32 ± 14 %). The HR and sNSA responses to PACAP-38, following pretreatment with PACAP6-38, were significantly attenuated compared with previous reports of PACAP-38 effects. We conclude that in the urethane anaesthetised, vagotomised, adult male Sprague-Dawley rat, endogenous PACAP is not tonically released onto receptors at the level of the spinal cord. The attenuation of the PACAP-38 response observed following pretreatment with PACAP6-38 indicates that the changes in sNSA and HR elicited by IT PACAP are mediated primarily via the PAC1 receptor.
Figure 1: Synchrotron angiogram depicting non-uniform acetylcholine induced vasodilation of coronary vasculature in diabetic rat after NOS and COX blockade.

NITROXYL (HNO), A NOVEL REDOX SIBLING OF NITRIC OXIDE (NO\textsuperscript{\textdagger}), WITH VASOPROTECTIVE ACTIONS

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The therapeutic utility of the NO\textsuperscript{\textdagger}/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\textsuperscript{\textdagger} donor formulations is limited, however, by susceptibility to inactivation by superoxide (O_2^-) and tolerance development, properties which confer potential for the treatment of heart failure. Importantly, we have also demonstrated that HNO 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, HNO donors such as Angeli’s salt and IPA/HNO target predominantly the soluble guanylyl cyclase (sGC)/cGMP signaling pathway to mediate vasodilation. Moreover, in contrast to GTN, HNO donors do not develop vascular tolerance. Recently, our studies have shown that HNO donors limit oxidative stress, lowering vascular NADPH oxidase-derived O_2^- production in isolated intracranial arteries by ~65%. Such an effect is resilient to the NO scavenger, ODQ and absent in Nox2 deficient mice, suggesting that HNO donors modulate Nox2-containing NADPH oxidase possibly via a direct, cGMP-independent action. In addition, we have provided the first evidence that the vasoprotective actions of HNO are preserved in disease with the ability of HNO donors to induce vasorelaxation and inhibit platelet aggregation sustained in the setting of hypertension (SHR rats) and hypercholesterolemia (Apoe\textsuperscript{-/-} mice). In conclusion, HNO 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-isofrom specific arginine 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 (ArgiKO) mice were mounted in myographs and cumulative concentration-response curves (CRCs) to the NO donors, glyceryl 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). In contrast, tolerance to GTN (n = 6, P = 0.2) and SNP (n = 6, P = 0.05) was not evident in ArgiKO mice. In vivo GTN tolerance was induced by injecting GTN (20 mg/kg s.c.) 8 hours for 3 days. A vehicle (5% glucose) control was used. Tolerance was evident in GTN treated WT aorta with a 7-fold shift in the CRC (Vehicle: EC\textsubscript{50} = 8.8 \pm 2.0, vs GTN: EC\textsubscript{50} = 81.3 \pm 0.3, n = 6, P < 0.05). In contrast, tolerance to GTN (n = 6, P = 0.05) and SNP (n = 6, P = 0.2) was not evident in ArgiKO mice. In vivo GTN tolerance was induced by injecting GTN (20 mg/kg s.c.) for those with a reduced nephron endowment. At 20 days of age WT (n = 27) were assigned to receive normal (0.26%) or high salt (5% NaCl) diet. At 10 weeks of age mice underwent 24-h urine collection for measurement of GFR (creatinine clearance) and then had 24h blood pressure measured by radiotelemetry. Following recordings, hearts and kidneys were excised and abdominal aorta and femoral arteries were collected for assessment of wall stiffness. Bodyweights were unaffected by high salt diet, but kidney weight, kidney to bodyweight ratio and left ventricle to bodyweight ratios were all significantly (P < 0.01) greater in mice on a high salt diet. Mean arterial pressure and GFR were not different between WT and GDNF Het mice on normal salt diets. GDNF Het and WT mice on high salt diets had GFR values ~75% greater than normal salt controls but blood pressures were not different between groups. Wall stiffness in arteries of WT and GDNF Het mice on normal salt were not different but markedly increased (P < 0.0001) on high salt diet. Further, in a subset of WT mice (n = 4) in which the high salt diet was switched to a normal salt diet for 5 weeks, the enhanced stiffness was not reversed. High salt diet initiated at weaning lead to marked kidney and cardiac hypertrophy, renal histological, fibrosis and vascular stiffening in young mice that was not reversed after 5 weeks of normal salt diet. These changes occurred in the absence of hypertension. These findings suggest that greater emphasis should be placed on reducing salt content in food targeted at children and that blood pressure may not be an adequate early marker of cardiovascular risk for those on a high salt diet.

HIGH SALT DIET DURING EARLY POSTWEANING LIFE LEADS TO ADULT CARDIOVASCULAR DYSFUNCTION IN ABSENCE OF HYPERTENSION

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Adult consumption of dietary salt is well in excess of need and has been associated with adverse cardiovascular outcomes. Alarmingly the intake of dietary salt by young children is also excessive due to the intake of salty snacks. The current study aimed to examine the impact of high salt diets initiated in weaning period for adult cardiac dysfunction in mice. GDNF Heterozygous mice were also subjected to high salt diets to determine whether outcomes would be worsened for those with a reduced nephron endowment. At 20 days of age WT (n = 33) and GDNF Het mice (n = 27) were assigned to receive normal (0.26%) or high salt (5% NaCl). At 10 weeks of age mice underwent 24-h urine collection for measurement of GFR (creatinine clearance) and then had 24h blood pressure measured by radiotelemetry. Following recordings, hearts and kidneys were excised and abdominal aorta and femoral arteries were collected for assessment of wall stiffness. Bodyweights were unaffected by high salt diet, but kidney weight, kidney to bodyweight ratio and left ventricle to bodyweight ratios were all significantly (P < 0.01) greater in mice on a high salt diet. Mean arterial pressure and GFR were not different between WT and GDNF Het mice on normal salt diets. GDNF Het and WT mice on high salt diets had GFR values ~75% greater than normal salt controls but blood pressures were not different between groups. Wall stiffness in arteries of WT and GDNF Het mice on normal salt were not different but markedly increased (P < 0.0001) on high salt diet. Further, in a subset of WT mice (n = 4) in which the high salt diet was switched to a normal salt diet for 5 weeks, the enhanced stiffness was not reversed. High salt diet initiated at weaning lead to marked kidney and cardiac hypertrophy, renal histological, fibrosis and vascular stiffening in young mice that was not reversed after 5 weeks of normal salt diet. These changes occurred in the absence of hypertension. These findings suggest that greater emphasis should be placed on reducing salt content in food targeted at children and that blood pressure may not be an adequate early marker of cardiovascular risk for those on a high salt diet.

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 on sequential cardiac cycles (N = 67) of simultaneous waveforms using applantation tonometry and transcranial Doppler respectively in a single animal experiment. The aorta was isolated and the distal aortic arch was cannulated and distal pressures and flows were recorded forward 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 were evaluated in the present study. The average time lags between pressure and flow signals were 10.7, 13.0, 15.0, 17.0, 25.0 and 50.0 msec, respectively, in N = 30 animals. The CCP was highest in the negative pressure region with mean and SD of 41.7 \pm 3.1 mm Hg and then decreased to a minimum of 28.8 \pm 3.2 mm Hg at 50.0 msec. RAP was highest in the negative pressure region with mean and SD of 2.1 \pm 0.1 mm Hg and then decreased to a minimum of 1.6 \pm 0.1 mm Hg at 50.0 msec. The time lags between pressure and flow signals had a significant effect on the calculation of CCP and RAP. The time lags between pressure and flow signals had a significant effect on the calculation of CCP and RAP.
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.

### 046 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 neurohumoral block (NHB), which eliminated activity of the ANS and the pressor hormones Ang II and AVP. As agonists we infused AngII, methoxamine, acetyl choline and adenosine, to derive extended scaled dose (ScD) – total peripheral conductance (TPC) and – TPR response curves. Earlier only the TPC curves were examined, and the slope ratios (H/N) between hypertensive and normotensive animals determined by linear regression. As a result we missed two major non-linearities that were more obvious in the TPR curves. On this occasion both curves were examined by polynomial regression, which accounted for 85–95% of their variance. The non-linearities were due to: 1) functional rarefaction associated with reduction of CO at high doses of constrictors; 2) hypotension caused by high doses of dilators, which limited autoregulatory capacity. Between these non-linearities there remained a substantial dose-range for assessing the H:N ratios (H:N) between hypertensive and normotensive animals determined by linear regression.

### 047 THE BENEFITS OF A FIXED COMBINATION OF PERINDOPRIL AND INDAPAMIDE ON CARDIOVASCULAR OUTCOMES IN PATIENTS WITH TYPE 2 DIABETES AND CHRONIC KIDNEY DISEASE

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ACE-inhibitors and diuretics have been shown to reduce blood pressure and albuminuria; both important risk factors for kidney disease progression. Since individuals with chronic kidney disease (CKD) are at substantially increased risk for cardiovascular disease, the benefits of these agents could be particular large in this population. We therefore assessed the effects of combination ACE inhibitor-diuretic therapy according to the degree of CKD. Data from the ADVANCE trial was used to assess the effects of a fixed combination of perindopril-indapamide (4 mg/1.25 mg) or placebo on major macrovascular events (cardiovascular death, myocardial infarction or stroke) in subgroups according to baseline Kidney Disease Outcome Quality Initiative defined CKD stage. Statistical tests for interaction were performed in the relevant Cox models to test for homogeneity in treatment effect across CKD stages. The ADVANCE trial included 11,140 participants of which 6625 had no CKD, 2482 were classified as CKD stage 1 or 2 and 2033 as CKD stage ≥ 3. The relative risk reductions (RRR) and Numbers Needed to Treat (NNT) according to CKD stage are presented in Table 1. There was no heterogeneity in the magnitude of the treatment effects for each outcome across the subgroups (all P<0.18). The absolute treatment effects were markedly higher in those with CKD ≥ 3 as compared to those with no CKD. For every 1000 patients with CKD stage ≥ 3 treated for 5 years, active treatment prevented 12 macrovascular events, 16 cardiovascular deaths and 20 all-cause deaths, as compared to 6 macrovascular events, 6 cardiovascular deaths and 10 deaths per 1000 patients with no CKD. The reductions in the relative risk of vascular outcomes and death with perindopril-indapamide in patients with type 2 diabetes were similar across subgroups defined by stage of CKD at baseline. As expected, the absolute treatment effects were greater in patients with CKD, highlighting the importance of blood pressure lowering in this population.

### 048 EFFECTS OF GHRELIN INFUSION ON SYMPATHETIC NERVOUS SYSTEM ACTIVITY IN HUMANS

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Ghrelin is a recently discovered GH-releasing peptide secreted by the stomach with effects on appetite and cardiovascular regulation. Animal studies suggest that ghrelin acts centrally to decrease the activity of the sympathetic nervous system (SNS) and reduce cardiovascular mortality by mediating behavioural responses to stress. To investigate the effects of ghrelin in humans, we gave 9 lean healthy men (age 21±0.3 years) an intravenous infusion of human ghrelin (5pmol/kg/min for 1 hour) and saline in a randomized fashion. Ghrelin elicited a small decrease in systolic and diastolic blood pressure (−1.12±0.3 mmHg and −1.12±1.1 mmHg vs −4 ± 4 mmHg for diabetic, during ghrelin and saline infusion respectively, P<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 microneurography (from 18±2.5 to 27±4.4 bursts per min, P<0.005) while no change occurred during saline infusion (from 18.2±2 to 17.1±2.1 bursts per min). Ghrelin, but not saline, induced a rise in plasma glucose concentration (from 4.4±0.1 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 MSNA during blood 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 vasodilatory effect rather than by affecting the central nervous system. Furthermore, ghrelin may contribute to the stress induced cardiovascular responses.

### 049 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 reappearance of apoptosis mediates the cardioprotective effect of MR blockade. Sprague Dawley (SD) rats were anesthetised, 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 to inhibit inducing ischaemia and maintained throughout the reperfusion period. At the completion of reperfusion, infarct area and apoptotic cardiomyocytes (5.4±0.6%, N=10) were reduced during IR (1.0±0.3, N=10) and the number of TUNEL-positive cardiomyocytes (5.4±0.6%, N=10) were reduced during IR (1.0±0.3, N=10). Consistent with the EPL, spironolactone superfused alone significantly reduced infarct size (35±2%, N=8 vs 41±1%, N=7, P<0.05) and the number of TUNEL-positive cardiomyocytes (5.4±0.6%, N=7 vs 7.7±0.6%, N=7, P<0.05) via inverse agonist activity at mineralocorticoid receptors, an effect near-maximal at relatively low dose (10 nm). Although eplerenone (100 nm and 1 µM) reduced infarct size (mean values of 37±2%, N=9 and 36±2%, N=8, respectively) this was not significant (P=0.10 & 0.09). Consistent with the reduction in TUNEL staining, spironolactone reversed both active caspase-3 and acinus processing during IR. Spironolactone also regulated anti-apoptotic protein ARC (apoptosis repressor with a caspase recruitment domain) activity. ARC expression was significantly reduced during IR (1.0×10⁶±0.9, N=7 versus 2.2×10⁶±0.3, N=7, P<0.05) and restored.

Calculated CCP and RAP values are essentially insensitive to relative time lags between pressure and flow signals in the range of –30 to 20 msec.
by 10 mM spironolactone (1.9 ± 0.01 ± 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-apoptotic protein(s).

050 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 to achieve metabolic enzymes, and during incorporation into plasma lipid fractions. This has the potential to manipulate the ratio of dietary n-6:n-3 PUFAs. In this study, there is a rational approach to the influence of the n-6:n-3 ratio on vascular function and lipoprotein profile. Subjects treated with statins remain at an increased risk of cardiovascular disease, and may particularly benefit from such dietary intervention. Subjects treated with statins (n = 6–8) received two dietary interventions with a different n-6:n-3 ratio (Diet A: 1.71 and Diet B: 3.01) in a randomised, crossover fashion. Measures of lipid profile, blood pressure, brachial compliance and distensibility, and pulse wave velocity were determined. Data are presented as mean ± standard error mean using paired t-tests. Both diets caused significant reductions in total cholesterol (Diet A: 4.3 ± 0.7 to 4.5 ± 0.8 mmol/L, n = 6, P = 0.04; Diet B: 3.9 ± 1.5 to 5.0 ± 1.2 mmol/L, n = 8, P = 0.02) and LDL cholesterol (Diet A: 2.9 ± 0.5 to 2.4 ± 0.5 mmol/L, n = 6, P = 0.01; Diet B: 3.6 ± 0.5 to 2.7 ± 0.5 mmol/L, n = 8, P = 0.007). We also observed a significant reduction in systolic and diastolic blood pressure (Systolic: 123.7 ± 6.5 to 113.6 ± 6.8 mmHg, n = 6, P = 0.01; Diet B: 5.9 ± 1.5 to 112.7 ± 6.9 mmHg, n = 8, P = 0.02) in subjects who received the diet with low n-6:n-3 ratio (Diet A). Other parameters were not affected by either diets (P > 0.10). These results suggest that dietary intervention can markedly reduce LDL cholesterol in patients treated with statins.

051 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 at 0.2 and 1.3 mmol/L, n = 4–9) received two different n-6:n-3 ratios (Diet A: 1.71 and Diet B: 3.01) in a randomised, crossover fashion. Measures of lipid profile, blood pressure, brachial compliance and distensibility, and pulse wave velocity were determined. Data are presented as mean ± standard error mean using paired t-tests. Both diets caused significant reductions in total cholesterol (Diet A: 4.3 ± 0.7 to 4.5 ± 0.8 mmol/L, n = 6, P = 0.04; Diet B: 3.9 ± 1.5 to 5.0 ± 1.2 mmol/L, n = 8, P = 0.02) and LDL cholesterol (Diet A: 2.9 ± 0.5 to 2.4 ± 0.5 mmol/L, n = 6, P = 0.01; Diet B: 3.6 ± 0.5 to 2.7 ± 0.5 mmol/L, n = 8, P = 0.007). We also observed a significant reduction in systolic and diastolic blood pressure (Systolic: 123.7 ± 6.5 to 113.6 ± 6.8 mmHg, n = 6, P = 0.01; Diet B: 5.9 ± 1.5 to 112.7 ± 6.9 mmHg, n = 8, P = 0.02) in subjects who received the diet with low n-6:n-3 ratio (Diet A). Other parameters were not affected by either diets (P > 0.10). These results suggest that dietary intervention can markedly reduce LDL cholesterol in patients treated with statins.

052 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 mechanistic 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 (MRC) –

epithelial sodium channels (ENaC) – Na⁺/K⁺-ATPase have now been demonstrated to be present in the central nervous system. This pathway is being regulated independently of the peripheral/renal pathway and contributes to regulation of cerebrospinal fluid [Na⁺] by the choroid plexus, of brain tissue [Na⁺] by the ependyma and to neuronal responses to e.g. Na⁺ or Ang II. Increases in CSF [Na⁺] by central infusion of Na⁺-rich aCSF or by high salt intake in Dahl S or SHR cause sympathetic activation and hypertension. These responses appear to depend on activation of a CNS cascade starting with aldosterone – MiRNAC – “ouabain”, the latter lowering neuronal membrane potential leading to enhanced Ang II release in e.g. the PVN. Specific CNS blockade of any of the steps in this cascade from aldosterone synthase blockade to AT1-receptor blockade prevents the sympathetic hyperactivity and hypertension on high salt intake, irrespective of the presence of a “salt-sensitive kidney”. We propose that in salt-sensitive hypertension an increase in CSF [Na⁺] causes a local increase in aldosterone biosynthesis which activates an aldosterone dependent neuromodulatory pathway which enhances activity of anti-angiotensinergic sympatho-excitatory pathways leading to hypertension. Looking beyond the kidney is providing new insights into mechanisms contributing to salt-sensitive hypertension, which will help to dissect the genetic factors involved and to discover novel strategies to prevent and treat salt-sensitive hypertension.

053 LARGE ARTERY STIFFNESS INCREASES WITH LOCAL NEUROGENIC BLOCKADE IN RATS

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Whilst the functional role of neurogenic control of peripheral blood vessels is well established, the presence of neurogenic terminals in the large arteries has not been linked with changes in large artery stiffness. The aim of this study was to elucidate any changes in rat arterial stiffness, through measurement of pulse wave velocity (PWV), as a result of local neurogenic blockade. Methods. Male Sprague-Dawley rats (n = 7, aged 12-14weeks) were anaesthetised and two 1.3F, high fidelity pressure sensors introduced into the proximal and distal ends of the abdominal aorta for measurement of PWV. Increasing doses (1ug/ml, 10ug/ml & 100ug/ml) of an alpha-adrenergic antagonist (phentolamine) were used to locally chemically denervate the abdominal aorta via bathing through a ventrally exposed cavity. Mean arterial pressure was raised and lowered using intravenously injected phenylephrine and sodium nitroprusside respectively. PWV was measured across the full physiological arterial pressure range, and during the different levels of local neurogenic blockade. Results. The increasing concentrations of phentolamine caused aortic PWV to increase concordantly. Neurogenic blockade caused a significant change in PWV at arterial pressures above 110mmHg, with PWV increasing from 5% (1ug/ml phenylephrine) to 15% (10ug/ml phenolamine) compared to controls (P < 0.05). The response was more pronounced at aortic pressures above 130 mmHg, with 10ug/ml phenolamineresulting in a 12.5% increase in PWV compared to control (P < 0.01). Conclusions. These results show that local neurogenic blockade in large arteries can significantly increase local arterial stiffness. This demonstrates that neurogenic input into large arteries results in functional physiological changes that could impact on blood pressure regulation.

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

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Exposure to high energy radiation causes cardiovascular injury. It has been demonstrated that whole-body gamma irradiation impairs the endothelial-dependent vasomotor function. Such impairment may cause increased arterial stiffness, as can be shown by increased pulse wave velocity (PWV). The current study aims to determine the effects of whole-body high-dose high-energy X-ray on aortic PWV. Male Sprague-Dawley rats (14 weeks old) were exposed to a single high-dose of high-energy X-ray (source: LINAC, 6 MV) at either 0 (control) or 5 Gy (irradiated). Between 0 to 24 hours, 24 to 48 hours and 3 weeks following irradiation, rats were anaesthetized (1.3 g/kg, urethane) and beat-to-beat PWV (m/s) and mean arterial blood pressure (MAP) measured invasively using two 1.3F high fidelity pressure sensors (SiSonde Inc, Canada) positioned in the descending thoracic aorta via the right femoral and left carotid arteries. Arterial pressure was increased and decreased over the range of 60 –150 mmHg with intravenous infusion of phenylephrine and sodium nitroprusside respectively. Across the blood pressure range, mean aortic PWV in the irradiated rats measured within 24 hours post-irradiation was 5.0±0.15 m/s, a 14% increase compared to the control group (4.4±0.11 m/s, n = 12, P < 0.05). No significant difference was observed between the PWV in the irradiated group measured in the 24–48 hour period post-irradiation and the control group (4.14±0.10 m/s, n = 4, P < 0.04). Similarly, no difference was found in the arterial stiffness of control and irradiated rats 3 weeks after radiation (control mean ± SE: 4.37±0.14 m/s irradiated – 4.53±0.13 m/s, n = 12; P = 0.41). Whole-body exposure to a single dose of high-energy X-ray increased aortic stiffness in the period of 0 to 24 hours after the radiation treatment. These changes were reversed in the subsequent 24 hour period, and remained so 3 weeks following irradiation. These findings suggest an acute mechanism behind endothelial impairment associated with whole-body, high-dose irradiation. Further research is required to ascertain the exact nature of that mechanism.
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 intravascular tissues and amniotic fluid contain prorenin. Amniotic (pro)renin can generate Ang I from angiotensinogen (AGT) or stimulate cell signalling pathways directly when bound to the (pro)renin receptor (P)RR. To measure the expression of RAS components, including prorenin, (P)RR, AGT, ACE1, ACE2, AT1R, AT2R, MAS1, amnion, 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 decidua prorenin mRNA in pregnancies carrying a female fetus (P=0.035). (P)RR mRNA was highest in placenta (P<0.001). AT1R mRNA was highest in decidua (P<0.003). ACE1 mRNA was lowest in fetal membranes (P<0.001) and higher in decidua than in placenta (P<0.003). ACE2 mRNA was lowest in fetal membranes (P<0.001) and highest in placenta (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 amnion, mRNA expression of RAS components is low, but (P)RR is abundant and may bind protein from amniotic fluid or decidua. In decidua, RAS components are abundant (except AT1R). In placenta, since ACE protein is localized to fetal capillary endothelial cells and AT1R and AT1R protein are present, 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 maternal HDP. The average SBP after 1 hour of observation, if the first SBP reading was discarded, did not significantly differ from the average SBP after 2, 3 or 4 hours of observation. However, 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 reparative mechanisms that occur in the brain after stroke. Angiogenesis in the damaged brain is crucial to support surviving 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 following ischemic stroke. We have also identified the temporal and oxygenic signalling factors involved in this response in rats. Functional deficits in behaviour were detected between 1 and 7 days post-stroke (P<0.01). Blood vessel numbers decreased within the cortical infarct core 6 h after stroke (30±10% P<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 vessels in the infarct core at 14 and 28 days was associated with DHE-detectable superoxide generation and only occurred in brain regions that had lost all neurons. Real-time PCR detection of NADPH oxidase (Nox) subunits revealed a marked increase in Nox2 mRNA expression in the ipsilateral cortex up to 7 days post-stroke (up to 40 fold P<0.001) which returned to normal by 14 days. Nox4 mRNA was significantly increased later at 14 days. Angiogenic factor VEGF mRNA increased and blood vessel numbers increased between 7 and 14 days post-stroke (2–3 fold P<0.05) but returned to normal levels by 28 days. Transplantation of stem cells into the brain post-stroke is currently thought to be an exciting treatment option. Optimisation of the damaged brain environment after stroke is crucial for the survival of transplanted cells. NADPH oxidases may be involved in the regulation of angiogenesis in the brain in the weeks following ischaemic stroke and reperfusion and therefore present a target for enhancement of brain repair.

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

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The result of the modern style of life (lack of physical activity, surplus use of high 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 20–65%. In this group of people the level of death is twice higher and level of cardiovascular symptoms three times higher than in patients without metabolic syndrome. In patients with arterial hypertension, function of the renal system decreases by 1% per year, and arterial hypertension together with diabetes – by 13%. The aim of the study was to compare the influence of anti-hypertension therapy on the speed of glomerular filtration. There were 39 patients with metabolic syndrome 33–83 years old. Patients underwent the following examination that measured: the body index, circumference of the waist, circumference of the thigh, sagittal size of the stomach, percentage of body fat, arterial heart rate, blood sugar, triglycerides and general cholesterol in blood, echocardiography in B and M regimes, creatinine level and creatinine clearance, and the glomerular filtration rate (GFR). Patients were divided into two groups, and they were all treated with standard therapy for metabolic syndrome, including hypoglycemic medication and statins. Groups received different antihypertensive therapy. 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 70.1±3.63 ml/min to 86.37±11.53 ml/min (n=19; P<0.05). 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 blockade is more effective for increasing GFR than angiotensin converting enzyme inhibition.

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

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Endothelial progenitor cells (EPC) are primitive cells that are important in endothelial repair and regeneration in blood vessel walls following injury. Their impairment is associated with cardiovascular disease (CVD) and risk factors. It is well regarded in non-obese populations that EPC levels serve as a cellular biomarker of CVD. However, EPC level and role in severe obesity are yet to be established. To explore whether EPC could function as a cellular biomarker of CVD in the severe obese, a correlation study was carried out. In this study we assessed EPC number.
and function andcarotid intima-media thickness (IMT), an established non-invasive marker for atherosclerosis. In 63 severe obese subjects (46.3±1.1 yrs, BMI 45.2±0.7 kg/m²), and 26 age and gender-matched controls (48.1±2.3 yrs, BMI 25.5±0.5 kg/m²). Circulating EPC level was determined by FACS counting of percentage of AC133+/KDR- cells in 10⁵ Ficoll-density isolated peripheral mononuclear cells, and EPC-colony forming unit (EPC-CFU) was assessed using a standard EPC-CFU assay. EPC number and EPC-CFU were increased in 1.7-fold (0.089±0.011% versus 0.051±0.011%, P<0.04) and 3.4-fold (11.1±1.9 colonies/well vs 3.3±1.1 colonies/well, P<0.03) respectively, in the severely obese compared to controls. IMT was greater in the severely obese subjects (0.658±0.016 mm vs 0.570±0.016 mm, P<0.001) compared to controls. Correlation analysis revealed that EPC number was positively associated with IMT in the controls (P<0.02) but not in the obese while EPC-CFU did not show any correlation. These results indicate that in severe obesity there is no impairment of EPC function, suggesting that EPC are not an adequate cellular biomarker of CVD in this population. These findings illustrate the complexity of the pathophysiology in the severe obese. Meanwhile the ethanol exposure during pregnancy, whilst having minor effects on basal blood pressure, ethanol when injected into Merino ewes prior to mating. Vascular catheters were implanted at 120–130d gestation in 6 in each group. Calcification was induced in 8 weeks old WKY with vitamin D3 injection and the second after 8 hours. Using simultaneous catheterization of proximal and distal aortic sites in Wistar Kyoto (WKY), spontaneously hypertensive (SHR) and calcified (VDN) rats. Experiments were performed in anaesthetised (Urethane, 1.3 g/kg, ip) WKY, age matched SHR and VDN rats (n=6 in each group). Calcification was induced in 8 weeks old WKY with vitamin D3 (30000 IU/kg, im) and 2 doses of nicotine (25 mg/kg, p.o.), one with vitamin D3 injection and the second after 8 hours. Using simultaneous catheterization of proximal and distal aorta with two high fidelity 1.4F catheters, aortic beat-to-beat EPP was recorded over a wide range of BP. MAP was increased and decreased by 30 second intusions of phenylephrine (50 μg/min) and sodium nitroprusside (10 μg/min). PP was defined as the ratio of the PP recorded at the distal sensor to the proximal. PPA of VDN was attenuated to that of SHR but did not converge to WKY suggesting intrinsic differences between the SHR and WKY. PPA of SHR up to 120 mmHg compared to WKY then converged to unity. PPA of VDN was attenuated to that of SHR but did not converge to WKY suggesting intrinsic changes might have occurred in the vascular properties.
REDUCED ANGIOTENSIN GEN EXP FOLLOWING ANGIOTENSIN II STIMULATION OF ASTROCYTES EXPRESSING THE ANGIOTENSIN TYPE 1A RECEPTOR

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Angiotensin II (AngII) acts via AT₁ receptors in several brain regions to regulate blood pressure – one site is in the rostral ventrolateral medulla (RVLM) where AngII directly excites sympathetic premotor neurons. Increased angiotensin type 1A (AT₁A) 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 AT₁A receptor activity in astrocytes might increase Ao production, leading to increased local AngII production and sympathoexcitation. Positive feedback regulation of Ao production by AngII has been demonstrated in vitro. 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 eliciting 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 receptor 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 2005. Only Concessional patients were included because many AHT products fall under the General copayment. Initiation was defined as 2 consecutive months of an ACE and a DHP following at least 6 months without an ACE/DHP combination. Treatment cessation was 3 consecutive months of none or just one of the drugs making up the combination. Hazard ratios were derived and adjusted for patient age, sex and initiating specialty. Hazard ratios were derived and adjusted for patient age, sex and initiating specialty. More than 9,500 patients initiated on a DHP/ACE combination, had their persistence to the combination assessed. Median persistence differed between combinations: lercanidipine/A2RA 23 months [22–25], felodipine/A2RA 21 months [19–24], nifedipine/A2RA 16 months [14–18], and amlodipine/A2RA 15 months [14–17]. Using lercanidipine/ACE as the reference, patients were more likely to cease the other combinations felodipine/ACE (7.2%, P<0.08), nifedipine/ACE (26.5%, P<0.05) and amlodipine/ACE (26.6%, P<0.05). Prescribers need to assess which DHP best supports NHF treatment goals. In terms of optimal treatment persistence, lercanidipine seems to be the best DHP to combine with an A2RA.
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 AT1MUT higher expressed BP and heart rate (HR) levels compared to controls (systolic BP AT1MUT 134.6 ± 5.7 mmHg; AT1WT 110.5 ± 5.6; P = 0.05; HR AT1MUT 53 ± 15, AT1WT 45.5 ± 5.7 beats/min; P = 0.001). Systolic BRS was diminished in both genotypes compared to the respective control, AT1MUT 1.33 ± 0.17 ms/mmHg; AT1WT 1.91 ± 0.18 ms/mmHg; P = 0.05). Motor activity did not differ between groups. These variables exhibited circular 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 and the lowest values in the daytime. In all cases, 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 sympathetic vasomotor function, was 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 contribution of central sympathetic vasomotor pathways and also inhibition of the cardiac baroreflex which may contribute to the hypertensive response.

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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 siblings reported five years ago revealed early cardiovascular 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 patients with genetically proven GSH who had been previously shown to have structural and functional changes on echocardiography (compared with 24 age- and sex-matched normotensive controls) were restudied after 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, LV mass remained similar (130.6 ± 12.7 vs 124.8 ± 15.8 mg; P = 0.5707) and diastolic BP increased (72.0 ± 9.6 vs 80.3 ± 14.5 mm Hg; P = 0.0178), LV posterior wall (0.83 ± 0.09 vs 1.01 ± 0.13 cm; P = 0.0185), LV mass (123.9 ± 19.5 vs 190.4 ± 53.7 g; P = 0.0031), LV mass index (72.4 ± 8.7 vs 103.6 ± 20.8 g/m²; P = 0.0102) and mitral inflow deceleration time (75.5 ± 30.7 vs 203.7 ± 35.6 ms; P = 0.0021) increased after follow-up. There were no significant differences in LV diameters and volumes, interventricular septum, ejection fraction, CVIB, E/A wave ratio and E/E’ ratio. In GSH, aldosterone excess is associated with increased LV wall thicknesses, LV mass and reduced diastolic function, suggesting that specific treatment (either partial ACTH suppression or aldosterone blockade) should be commenced early and perhaps even long before hypertension develops. Given possible growth-retarding effects of glucocorticoids in children, and lack of perfect receptor specificity of spironolactone, the time has come to discuss optimal treatment for children with GSH.

070

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

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Experimental and human data suggest that the cardiorenal effects of aldosterone excess are dependent upon concomitant dietary salt intake. Increased urinary protein (Uprot) is an early sign of nephropathy independently associated with cardiovascular risk. In a previous study we demonstrated that there is a positive association between Uprot and urinary sodium (UNa) in patients with hyperaldosteronism, but not in patients with normal aldosterone levels. The objective of the current study was to determine if Uprot is related to UNa in patients with hyperaldosteronism, but not in patients with normal aldosterone levels. The rapid response in some patients is consistent with a role for sympathetic vasomotor function, which in mice is an index of sympathetic vasomotor function, was 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 contribution of central sympathetic vasomotor pathways and also inhibition of the cardiac baroreflex which may contribute to the hypertensive response.

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. The aim of the current study was to determine if offspring from obese mothers had altered cardiovascular and sympathetic responses to ghrelin and stress. Female New Zealand White rabbits were fed either a control (3.5% fat) or high fat diet (HFD, 13.5% fat) for 3 weeks prior to mating and throughout gestation and lactation. After weaning, offspring were fed a restricted control diet. At 4 months of age all rabbits were instrumented with intracerebroventricular (ICV) cannulae and renal nerve electrodes. Body weight was similar between groups, however HFD offspring (n = 9) had heavier visceral white adipose tissue compared to control offspring (n = 8, P < 0.05). Offspring from HFD mothers had an elevated blood pressure, heart rate and renal SNA in comparison to control offspring (P < 0.05). Ghrelin administration (1civ, 1, 2, 4 & 5 nmol) dose dependently decreased blood pressure and heart rate in both groups but elevated renal SNA in HFD offspring only. While the cardiovascular and sympathetic responses to acute airjet stress were similar between groups, icv administration of ghrelin reduced the pressor, tachycardic and renal SNA response to stress in both groups. This inhibition of stress responses was less in offspring from fat fed mothers compared to control (P < 0.05). These studies show that the normal sympathoinhibitory actions of ghrelin are diminished in offspring from fat fed mothers leading to a greater reactivity to stress during periods when ghrelin may be released prior to eating.

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HIGH FAT FEEDING IN RABBITS ALTERS LEPTIN SENSITIVITY AND ELEVATES RENAL SYmpathetic NERVE ACTIVITY AND ARTERIAL PRESSURE

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The activation of the sympathetic nervous system (SNS) through the central actions of the adipokine leptin has been suggested as a major mechanism by which obesity contributes to the development of hypertension. However, direct evidence for elevated sympathetic activity has been limited to muscle. The present study examined the renal sympathetic nerve activity (RSNA) and cardiovascular effects of a high fat diet (HFD) as well as the changes in the sensitivity to intracerebroventricular (icv) leptin. New Zealand White rabbits fed a 13.5% HFD for 4 weeks showed modest weight gain but a 2–3 fold greater accumulation of visceral fat compared with control rabbits. Mean arterial pressure (MAP) and heart rate increased by +8% and +26% respectively and RSNA was +48% higher (P < 0.05) as was plasma non-epinephrine concentration (+35%, P < 0.05) following three weeks of HFD. Icv leptin administration (5–100 µg) increased MAP similarly in both groups but RSNA increased more in HFD fed rabbits. By contrast, icv leptin produced a dose dependently reduced RSNA in control rabbits in regions important for appetite and sympathetic actions of leptin (accurate ~54%, paraventricular ~69% and dorsomedial hypothalamus ~65%). These results suggest that visceral fat accumulation through consumption of a HFD leads to marked sympathetic activation, which is related to increased responsiveness to central sympathoexcitatory actions of leptin. The paradoxical reduction in hypothalamic neuronal activation by leptin suggests a marked ‘selective leptin resistance’ in these animals.

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OFFSPRING FROM HIGH FAT FED MOTHERS DISPLAY A SIMILAR CARDIOVASCULAR PHENOTYPE TO DIET INDUCED OBESE ABDUCTIONS

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We have previously demonstrated that offspring from rabbit dams fed a high fat diet (HFD) have elevated mean arterial pressure (MAP), heart rate (HR) and renal sympathetic nerve activity (RSNA) compared to control offspring at 4 months of age. One mechanism by which exchange to a HFD during development may contribute to the development of hypertension is through an elevation in visceral white adipose tissue (WAT) accumulation. The purpose of the present study was to compare RSNA, MAP, HR and WAT accumulation in adult New Zealand White Rabbits currently consuming a HFD, to those that were exposed to a HFD during development only (gestation and lactation). Offspring from mothers fed a HFD had a higher MAP (+7%) and HR (+11%) compared to control (n = 10, P < 0.05), but a lower MAP (+3%) and HR (+11%) compared to HFD fed rabbits (n = 8, P = 0.05). A correlation analysis indicated a strong association between MAP and peripheral and mesenteric WAT (r = 0.66, P = 0.01) such that every 100g of visceral fat accumulation increased MAP by 16 mmHg. However, regardless of whether rabbits were exposed to a HFD during development or adulthood, RSNA was similarly elevated in both groups compared to rabbits fed a control diet throughout life (+17% and +14% respectively). A correlation analysis and there was no correlation of RSNA to WAT. Thus while the hypertension is closely related to the degree of WAT accumulation, the level of sympathetic tone to the kidney may be adversely programmed during development and maintained long
term regardless of diet post-weaning. This study highlights how maternal nutrition can detrimentally impact on cardiovascular risk factors in the next generation.

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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 mass, 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 chow (CC) or high-fat diet (HFD). HFD then transitioned with males. At weaning, female offspring from CC dams fed chow (CC) and offspring from H dams were fed chow (HC) or HFD (HH). Half of each group were provided with a running wheel to enable voluntary exercise (CCex, HCex, HHex, n=10–12). Measurements included food intake, blood pressure and glucose tolerance. At week 14, brain, heart, muscle and fat were collected for mRNA measurement of markers for appetite regulation, cardiovascular and glucose and lipid homeostasis. Plasma leptin, insulin, triglycerides (TG), adiponectin and nonesterified fatty acid (NEFA) were determined. HC offspring weighed 12% more than CC offspring (P<0.05). They had increased fat mass, plasma leptin and adiponectin (P<0.05; HC vs CC), which were exaggerated by postnatal HFD (HH vs HC; P<0.01). HD consumption increased plasma TG and NEFA with a doubling of food intake and 37% increase in body weight (HH vs HC; P<0.01). Distance travelled on running wheel did not differ across groups. While exercise had no impact in CCex, exercise reduced the detrimental effects of maternal obesity in both chow and HFD offspring. Exercise reduced fat mass, plasma NEFA and adiponectin in HCex 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 is programmed by HFD and reversed by postnatal exercise. 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.

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MICROGLIA ARE ACTIVATED IN THE HYPOTHALAMUS FOLLOWING MYOCARDIAL INFARCTION

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Following a myocardial infarction (MI), inflammatory cytokines are elevated in the brain, as well as in plasma, indicating that inflammation is occurring in the brain in addition to the periphery. Microglia are the immune cells in the central nervous system and produce cytokines when they are activated by an insult or injury. In the present study, we investigated whether MI in rats induces activation of microglia in the brain. We used immunohistochemistry to detect CD11b (clone OX-42) and morphological changes to identify activated microglia. Compared to control brains, MI had undergone surgical repair and this was confirmed by b-amyloid deposition 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.

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

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

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

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

Results: Among the 2856 Australian REACH participants, 70.1% (n=2002) had uncontrolled blood pressure (>130/80 mmHg) and 86.3% (2522) had been taking antihypertensive medication. LTB was 70.7% (1784). The major univariate predictors of LTB were gender, age, diabetes, hypertension, carotid plaque, cholesterol, BMI and congestive heart failure. The cardiovascular event rate in those people with LTB was 5.6% while those on medication and controlling BP had a 3.7% rate. Assuming a hypothetical blood pressure intervention is applied to the LTB group resulting in controlled blood pressure (<130/80 mmHg), 28 cardiovascular events (CV death, non-fatal stroke, and non-fatal MI) per 1000 people and 21 cardiovascular disease events including coronary heart disease intervention such as CABG, coronary angioplasty, carotid surgery, etc. per 1000 people could be prevented.

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

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THE POWER OF THE MORNING BLOOD PRESSURE SURE 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 way of measuring 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 (BPPow). We have examined the association of morning BPPower and heart rate and long term clinical outcomes in elderly hypertensive patients monitored using ambulatory blood pressure monitoring in ANBP2.

Methods: BP-Power was calculated using a double logistic fitting procedure from 712 ambulatory recordings from male and female subjects. Five-year follow-up (short-term) was conducted by study nurses and 10 year follow-up was conducted via record linkage with the National Death Index with mail and telephone follow-up of survivors. Cox-proportional hazards models were used to determine the association of 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 BP power 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: 0.85 – 2.35; P=0.17), with a similar observation for mean power mean power (OR 1.35; 95% CI: 0.82 – 2.23; P=0.23). Diastolic BP Power had no impact on survival (OR 1.03; 95% CI: 0.63 – 1.68; P=0.90) However for heart rate power the reverse was observed. Subjects with the lowest heart rate power had a 23% lower risk of death (OR 0.77; 95% CI: 0.48 – 1.47; P=0.29).

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

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

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Hypertension is a global public health problem of major magnitude; an estimated 30–40% of the adult population in the developed world suffers from this condition. Despite the availability of pharmacologic therapies, only half of treated patients are controlled to established targets. Renal sympathetic hyperactivity is a major factor in the progression of hypertension. Catheter-based renal denervation has recently shown promise in treating this target, and thus lowering blood pressure (BP). Renal denervation using a radiofrequency catheter (Symplicity Catheter System, Aridiand Inc., Palo Alto, CA, USA) was tested in patients with resistant hypertension (systolic BP ≥160mmHg on ≥3 anti-hypertensive medications, including a diuretic), Office BP and safety data were measured at baseline and at 1, 6, 12, 9 and 12 months post-procedure. Fifty patients were enrolled at 5 centers in Australia and the EU; 5 patients were excluded for anatomic ineligibility pre-procedure. Among treated patients, mean age was 58±9 yrs, 44% were female, 31% diabetic and 22% had coronary artery disease. Baseline office BP was 177±12 mmHg and systolic BP was 111±12 mmHg despite patients being on a mean of 4.7 anti-hypertensive medications. Baseline eGFR was 81±23 mL/min/1.73m2 and heart rate was 72±11/min. A preliminary analysis of this study was published in the Lancet (2009;373:1275–1281) demonstrating a substantial and sustained reduction in BP following the procedure. However, only 9 of these patients (5 months post) were followed for ≥12 months. These results are of considerable relevance given the theoretical potential for the BP-lowering benefits of the procedure to wear off over time due to the potential of re-growth of ablated nerve fibers.
Therefore, full 12-month safety and effectiveness data is critical to ascertain the net benefit of cathepsin-based renal denervation. This late-breaking presentation will report on full 12-month safety, BP-lowering efficacy and supportive mechanistic data on the complete patient cohort. This should provide definitive data on whether the BP-lowering benefits initially observed are sustained over the full 12-months of the study.

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

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Evidence from experimental studies suggest that renalase, a soluble FAD-dependent protein, is involved in blood pressure regulation, possibly via degradation of catechol-amines including noradrenaline. To investigate whether renalase is associated with blood pressure levels and/or indices of arterial stiffness 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. Radiotracir dilution methodology and arterial blood sampling was applied to measure whole blood noradrenaline (NA) spillover. Arterial plasma levels of renalase were measured by Western blot analysis using a monoclonal anti-renalase antibody and quantified using a gel documentation system (Bio-Rad Quantity One Software). Split half analysis of the hypertensive cohort according to systolic blood pressure levels (mean: 168±22 vs 156±5mmHg; P<0.001) revealed that mean arterial renalase levels were substantially lower in the group with higher systolic blood pressure (62±31 vs 125±82 arbitrary units; P<0.05), whereas whole blood NA spillover tended to be higher in the group with higher systolic blood pressure without reaching statistical significance (645±445 vs 407±168mmHg/min; P=0.12). Arterial renalase levels were higher (238±174 arbitrary units) and whole body NA spillover was lower (168±78ng/min) in the normotensive control subjects (mean systolic blood pressure: 123±77mmHg). P<0.05. Correlation analysis revealed an inverse relationship between arterial renalase plasma levels and systolic blood pressure for the entire cohort (r=−0.52; P<0.05). These data suggest that arterial plasma levels of renalase are inversely associated with systolic blood pressure in a cohort of patients with resistant hypertension. Whether this relationship in part can be explained by alleviated degradation of noradrenaline or whether alternative pathways are involved requires further investigation.

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

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

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

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Low serum insulin-like growth factor-1 (IGF-1) is an independent risk factor for cardiovascular disease and diabetes. IGF-1 levels are a nonredundant disease 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 yrs). Fasting glucose, insulin, lipids, albumin, creatinine, liver enzymes, cotinine, high-sensitivity C-reactive protein (hsCRP), reactive oxygen species (ROS), IGF-1, blood pressure (BP) and pulse wave velocity (PWV) were determined.

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

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

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Exaggerated exercise blood pressure (EEBP) predicts mortality. The mechanisms of this association are unknown but have been linked to increased central arterial stiffness and raised central blood pressure (BP) during low-stress physical activity. Spironolactone has anti-fibrotic 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 randomized to 3 months spironolactone (25 mg daily; n=57) or placebo (n=55). An EEBP was defined as brachial BP ≥190/105 mmHg (women) or ≥210/105 mmHg (men) during maximal exercise. Arterial stiffness was recorded by aortic pulse wave velocity (PWV). Brachial BP was estimated by sphygmnopomanometer 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), VO2max 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 VO2max (r=–0.29; P=0.003). Compared with placebo, spironolactone significantly reduced 24ABPM SBP (r=−0.38 to −0.73 versus 1.0±0.8 mmHg; P<0.004), maximal exercise brachial SBP (r=−0.3±0.2 to −0.5±0.11 mmHg; P=0.002) and maximal central SBP (r=−0.7±0.16 versus −0.1±0.13 mmHg; P=0.007) but did not change aortic PWV, VO2max or LV parameters (P>0.05 for all). Moreover, central SBP (r=−4.2±1.1 versus 0.7±1.3 mmHg; P=0.03) and the systolic-pressure-time integral (r=−0.23±0.47 versus −1.1±0.49 mmHg/s) were significantly reduced during low-stress exercise (P<0.05 for both), whereas low-stress central SBP was unchanged by spironolactone (r=−3.8±16 versus 1.0±13 mmHg/s; 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.
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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 be synthesized in the periphery and is beneficial in the regulation of cardiovascular function. Recently it has been suggested that hydrogen sulphide acts within the brain to reduce blood pressure. In the present study we have investigated the effects of microinjection of hydrogen sulphide donors and the effects of inhibiting endogenous hydrogen sulphide production on blood pressure (BP), heart rate (HR) and lumbar sympathetic nerve activity (LNSA) in anaesthetised Wistar-Kyoto rats. There is evidence for considerable cellular uptake of hydrogen sulphide (H2S) in the hypothalamus and the pressor region of the rostral ventrolateral medulla (RVL/M), areas known to have important cardiovascular regulatory functions. Rats were anaesthetised initially with inhaled isoflurane (1–3% in air), the femoral vein and artery were cannulated and the lumbar sympathetic nerve exposed and recorded. Anaesthesia was then maintained using urethane (1–1.5 g/kg iv) with supplemental doses as required (0.1–0.3 g/kg iv). Depth of anaesthesia was monitored by testing the pedal and corneal reflexes. The results show that bilateral microinjections (10nl/side) of the hydrogen sulphide donor hydrosulphide (H2S, 20–2000 pmol, n = 5) into the PWN did not significantly affect BP, HR and LNSA, compared to vehicle microinjections. When hydroxymaline (0.2–2 mmol, n = 5) or amino-oxyacetate (0.1–1 mmol, n = 5), inhibitors of cystathionine beta synthase, an enzyme responsible for the production of hydrogen sulphide, were administered into the PWN, neither drug significantly affected the cardiovascular variables measured compared to vehicle. In separate groups of rats in which the NaH2S (0.2–2000 pmol, n = 5), or the inhibitors, hydroxymaline (0.2–2 mmol, n = 5) and amino-oxyacetate (0.1–1 mmol, n = 5), were microinjected bilaterally into the pressor region of the RVL/M, there was no significant effect on BP, HR and LNSA compared to vehicle controls. At the end of each experiment the injection sites in the brain were confirmed by histology. The results suggest that hydrogen sulphide in the hypothalamic PVN or the RVL/M does not play a major role in the regulation of the cardiovascular system.

808 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 hours, following a relative decline in these events during the night. It is thought that the morning surge in blood pressure in pregnant women may provide part of the explanation for the increased risk of cardiovascular events in pregnant women during the night. 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 = 3) female baboons had a telemetry device (Data Science Inc, USA) implanted prior to pregnancy. We measured 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 mmHg systolic and 83±2 mmHg diastolic and morning BP was 139±4 mmHg systolic and 90±1 mmHg diastolic. The MSBP was 25±5 mmHg systolic (P = 0.004) and 7±3 mmHg diastolic (P = 0.044). The mean MSBP was 12.3±mmHg systolic and 7±2 mmHg diastolic for the non-pregnant baboons, and 10.2± mmHg systolic and 3.2±mmHg diastolic for the pregnant baboons (not significantly different, P = 0.699 for systolic, P = 0.310 for diastolic). 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.

809 TUMOR NECROSIS FACTOR ALPHA INDUCES PLACENTAL ANTIANGIOGENIC FACTORS IN PREGNANT BABOONS (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 increased placental expression of endoglin (Eng), another anti-angiogenic factor implicated in PE. Telomere shortening mid-trimester baboons were given an infusion via the median femoral vein of either recombinant monkey TNF-α (1.7 ng/kg/day (n = 3) or saline (n = 3)) for 2 weeks. Transabdominal chorionic villous sampling was performed at baseline and weekly during treatment to obtain a fine biopsy of the placenta. The tissue was snap frozen and stored at −80°C. RNA was extracted from the tissue using a commercial kit (RNeasy Miniprep, Qiagen, Aus). RNA (0.25 μg) was reverse transcribed to cDNA using Superscript III reverse transcriptase and random primers (Invitrogen, Aus). Eng and sFlt-1 gene expression was assessed using quantitative polymerase chain reaction (Cycler, Biorad, Aus) with normalisation to a housekeeping gene, 18S. Mann-Whitney U tests were performed for statistical analysis using PASW Statistics 17.0 (SPSS, Inc., USA). RNA of sufficient quantity and quality for qRT-PCR was obtained from n = 2 animals per group. To allow for statistical analysis, data from the 1 week of treatment timepoint and the 2 weeks of treatment timepoint were grouped for each animal. Average sFlt-1 gene expression was more than 20-fold greater in the treated animals compared to the control animals [2017 vs 100% relative expression, P < 0.014]. Eng gene expression was also significantly increased in the treatment group, with approximately 3-fold greater relative expression (373% vs 100% relative expression, P < 0.014). These results demonstrate that intravenous infusion of the pro-inflammatory cytokine TNF-α can induce placental production of the anti-angiogenic factors sFlt-1 and Eng which are associated with PE in humans. This study provides important evidence that a causal interaction between these two systems exists and may be critical in explaining the early placental pathophysiology in PE.

806 EXERCISE AUGMENTS WEIGHT LOSS INDUCED IMPROVEMENT IN RENAL FUNCTION IN OBSESE 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 interventions on renal function in this clinical setting and (2) correlates of improved renal function. Thirty eight MetS subjects (23.5 M, 15.9F; mean age 55±1 yrs: BMI 32.7±0.6 kg/m2) who fulfilled NCEP ATP III criteria were randomized to 12-weeks dietary weight loss intervention (WL and WL EX group) or control (CON). The WL and WL EX groups were treated with a daily dose of 2 g/d sodium saccharin and 0.6 g/d of noradrenaline, adrenomedullin/renin ratio and number of antihypertensive agents fell (P < 0.001), vital capacity (P < 0.01) and general health (P < 0.01). Compared with pre-ADX, there were significant increments in mean scores at 3 months for physical functioning (from 77.2±1 to 84.2±4, P < 0.001), role physical (83.7±1 to 86.1±4, P < 0.05), bodily pain (58.2±1 to 60.4±5, P < 0.05), social functioning (P < 0.05) and mental health (72.7±3 to 82.1±1, P < 0.001); and at 6 months for physical functioning (83.2±7 to 85.0±5), vital capacity (83.7±1 to 83.7±0.5), general health (75.7±0.0 to 80.5±6) and mental health (75.7±0.0 to 80.5±6). At 3 and 6 months the scores in each of the eight groups remained significantly improved from pre-ADX. 10 were in patients with unilateral PAL and (2) ADX has positive impacts not only on clinical and biochemical parameters but also on QOL, with significant QOL improvement observed as early as 3 months post-ADX and persisting at 6 months.

808 FACTORS IN PREGNANT BABOONS (PAPIO HAMADRYAS)

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Our study provides evidence that TNF-α is an inducer of hypertension in pregnancy, and suggests that the human placenta demonstrates evidence of a similar response to that seen in the baboon. These results suggest that baboon hypertension in pregnancy may reflect mechanisms similar to those demonstrated in the human. Future studies are required to determine if similar anti-angiogenic factors are released by the human placenta in pregnancy.
THE RECRUITMENT OF BLOOD-BORNE QDOT-Labeled CELLS INTO ATHEROSClerotic PLAQUE

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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 15mg/kg/day GSF 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 10th QDot-labeled autologous cells were injected weekly during this time. Viability of cells in vivo was confirmed the day after using flow cytometry. Labeling peptide, Ang (1–7) with, the ascending aorta and the coronary artery (LMA) were then studied by confocal microscopy. QDot-positive ‘neo’ endothelial cells were visible in both the ascending aorta and the LMA, which appeared to be concentrated on the shoulder of the plaque in the LMA. However, QDot-positive neo-intimal cells and medial cells were found only in the LMA and not the ascending aorta. There were also QDot-negative cells throughout both vessels. Conclusions: Blood borne CD45-negative cells form part of the neo-endothelial layer during an atherogenic diet, but only form neo-intimal and medial cells in the LMA at this time point. These studies strongly support the hypothesis that blood born cells can be recruited into atherosclerotic plaque, and that they appear to be concentrated in the ‘shoulder’ region of plaque.

HEART RATE-DEPENDENCE OF AORTIC PULSE WAVE VELOCITY IN RATS

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Aortic pulse wave velocity (aPWV), a surrogate measure of arterial stiffness, is a strong independent predictor of cardiovascular disease and all-cause mortality. Whilst studies have shown associated changes in arterial stiffness with acute changes in heart rate (HR), the effect of HR on aPWV at different mean arterial pressures (MAPs) have not yet been fully investigated. In this study, aPWV was measured in age-matched (12 weeks), urethane-anesthetised normotensive rats (n = 6) at different HRs across a wide MAP range (40 –160 mmHg). HR changes were achieved by atrial pacing at randomly sequenced rates (350, 400 and 450 bpm) after the resting HR was lowered with multiple doses of a bradycardic agent (zatebradine, 1mg/kg, i.v.). MAP was increased and decreased by infusions of phenylephrine and sodium nitroprusside respectively (30 μg/kg/min i.v.). Effects of HR on aPWV were analysed at each low (40 –80 mmHg), medium (80 –120 mmHg) and high (120 –160 mmHg) MAP range. Data are presented as mean ± se and Student’s 2-tailed F-test for paired observations was 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 HR. These findings indicate a HR-dependence of aPWV at high MAPs in rats. This is of particular interest as studies in humans have shown conflicting results of HR effects on arterial stiffness. These findings indicate a HR-dependence of aPWV at high MAPs in rats. This is of particular interest as studies in humans have shown conflicting results of HR effects on arterial stiffness. These findings indicate a HR-dependence of aPWV at high MAPs in rats. This is of particular interest as studies in humans have shown conflicting results of HR effects on arterial stiffness. These findings indicate a HR-dependence of aPWV at high MAPs in rats. This is of particular interest as studies in humans have shown conflicting results of HR effects on arterial stiffness.

ATHERO-PROTECTIVE EFFECTS OF THE ACE INHIBITOR, PERINDOPRIL, ARE PARTIALLY MEDIATED VIA ACTIVATION OF BOTH MAS AND AT2 RECEPTORS IN APOE3/LENTICULAR DEFICIENT MICE

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It is now well established that ACE inhibitors mediate athero-protective effects, both anti-hypertensive and athero-protective effects were reversed with co-infusion of either the Mas or the AT1 receptor antagonist. Restoration of ND bioavailability appears to play a significant role in the athero-protective effect mediated by perindopril treatment as eNOS immunoreactivity and protein levels were significantly increased whilst superoxide levels were significantly decreased, with both effects of perindopril reversed with either AT1 or Ma receptor blockade. In conclusion, we have shown for the first time that the athero-protective effects of the ACE inhibitor, perindopril are at least partially mediated by activation of both AT2 and Mas receptors, possibly involving stimulation by the angiotensin peptide fragment, Ang (1–7) that acts as a counter-regulatory pathway against the pro-atherogenic ACE/Ang II/AT1 axis.

VIRTUal Muscle provides Clues to the Protective Role of P3K in a Setting of myocardial Infarction

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It is now well established that cardiovascular disease can result from defects of components of the contractile apparatus. Recently, utilising cardiac-specific transgenic mice with a constitutively active (ca) mutant of phosphoinositide 3-kinase [P3K(p110α)] it was demonstrated that p3k(p110α) has a protective role in a setting of myocardial infarction (MI), in contrast, mice expressing the dominant negative receptor of P3K develop LV heart failure more rapidly in response to MI than control mice. Thus, activation of the P3K signalling pathway proves to be a potential therapeutic strategy for the treatment of heart failure; but the underlying molecular mechanisms remain elusive. Considering the range of cardiomyopathies that have been attributed to mutations of genes coding for structural proteins of cardiac muscle, the aim of this study was to identify associations between the protective effect of active P3K(p110α) and cardiac structural components. Here we have adapted a 3D Virtual Muscle (VMus3D) model to cardiac muscle that represents the location of these structural proteins for visualising micromarray data obtained from ventricles of non-infarcted ( sham) and MI operated caP3K and drP3K transgenic mice (relative to non-transgens). Using VMus3D for pattern recognition, a criterion was established that enabled us to select expression profiles that affected cardiac muscle structure according to P3K perturbation. Our approach has enabled us to draw on the entire gene expression dataset from a different perspective unbiased by highly differentially expressed transcripts. This has enabled us to identify mechanisms linking P3K to cardiac muscle structure regulation and glucose homoeostasis. In particular, we have identified mRNAs regulated by P3K perturbation under basal conditions that code for components associated with cardiac structure at the z-disc and costamere (Dag1, Ankrd26, Fln, Rock2, Cyr61 and Cdc45). We propose that the combined effects of these components may determine the heart’s ability to appropriately respond to mechanical stress in a setting of heart failure and provide a possible explanation and potential model (z-disc: costamere: hypertrophy axis) for how this could be operating.

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

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Resistant hypertension (HTN) is defined as blood pressure (BP) that remains above goal in spite of concurrent use of 3 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 combination with left ventricular dysfunction (LVH) 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. T2DM was defined as; male mean BMI ≤ 30 and female mean BMI ≤ 26, mean number of AHT 1.6 ± 3 and mean body mass index was 31.6 ± 6.4 kg/m². Subjects were divided into 4 groups: Gp A subjects were normotensive (BP <130/80) and on no AHT (n = 75, 6.2%); subjects in Gp B were at BP goal of <130/80 and on AHT (n = 195, 16.1%); Gp C subjects had not achieved BP goal of <130/80 and were on <3 AHT (n = 795, 58.1%), and Gp D subjects were classified as resistant HTN i.e. not at BP goal and were on ≥3 AHT (n = 209, 19.7%). Gp D had higher LV mass index and lower mitral annular early diastolic velocity (marker of LV relaxation) compared to the other groups (Figure 1). A significantly greater proportion of subjects in Gp D had LV diastolic dysfunction (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.
KYNURENINE IS A NOVEL ENDOTHELIUM-DERIVED VASCULAR RELAXING FACTOR IN ATHEROSCLEROSIS

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

AORTIC STIFFNESS, BUT NOT CENTRAL OR BRACHIAL BLOOD Pressures, Predict Physical Quality of Life

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Unless severe and uncontrolled, hypertension is commonly asymptomatic. However, some studies suggest that lower brachial blood pressure (BP) in patients with uncomplicated hypertension may improve quality of life (QOL). The relation between central BP and QOL has never been assessed, but may be relevant because large differences in central systolic BP (SBP) can occur between individuals with similar brachial SBP. This discrepancy between central and brachial SBP is mostly attributed to individual variation in large artery stiffness. We sought to determine the relation between QOL BP (brachial and central) and large artery stiffness in 104 patients receiving therapy for uncomplicated essential hypertension (aged 63 ± 2 years, 53% male) who were free from a history of cardiovascular or renal disease. The SF-36 health survey was used to quantify QOL. Brachial BP was assessed in the clinic (while seated and standing), at home (7-day average) and by 24 hour ambulatory monitoring (24ABPM). Central BP and aortic pulse wave velocity (PWV) (for arterial stiffness) were estimated by applanation tonometry. Neither brachial nor central BP’s were associated with QOL measures (P>0.05 for all). However, physical functioning scores significantly declined across tertiles of aortic PWV (tertile 1: 79 ±14, tertile 2: 76 ±22, tertile 3: 76 ±29) even after correcting for age, gender, clinic brachial SBP and 24ABPM SBP (ANCOVA P = 0.028). On multiple regression analysis, aortic PWV (but no BP measure) independently correlated with physical functioning (P = 0.026, P=0.012), but only accounted for 6% of the variance in this QOL analysis. We conclude that physical well being is negatively associated with large 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.

ANALOG STUDIES OF CHANGES OF ARTERIAL PRESSURE WAVEFORM WITH AGING

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Changes of features in cardiovascular parameters occur in different periods of life for populations. These changes can be investigated by means of physiological modeling, such as technique of multi-branched models to mimic the human arterial system. The aim of this study was to simulate the influence of aging on the shape of radial arterial pressure waveform. An aorta-radial transmission line model of the left arm was constructed and consisted of 11 segments from ascending aorta to radial artery through aortic arch, left subclavian, axilliary and brachial artery. Each segment was constructed by one electrical circuit representing viscous properties of blood (Ri), inertial properties of blood (Li), compliant properties of arteries (C) and leakages through small branches (RL). For simulation of increasing age, only Young’s modulus (E) of the conduit arteries was increased in a range of 10% while wall viscoelasticity (ηw) was set no change. Radial arterial pressure waveform was obtained from an aortic pressure waveform input in proximal and transfer function between them were calculated by means of FFT analysis. With increases in Young’s modulus, from 11.1 × 106 to 15.5 × 106 dyn/cm², the radial arterial waveform showed an earlier wave (wave velocity increase) and more sharp in ejection phase of waveform. The peak value of modulus of transfer function increased by 10% even after correction for age, gender, clinic brachial SBP and 24ABPM SBP (ANCOVA P = 0.028). On multiple regression analysis, aortic PWV (but no BP measure) independently correlated with physical functioning (P = 0.026, P=0.012), but only accounted for 6% of the variance in this QOL analysis. We conclude that physical well being is negatively associated with large 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.

COST BENEFIT OF SALT REDUCTION TO COMPLEMENT EXISTING CLINICAL HYPERTENSION PROGRAMS

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Excess dietary salt is a key contributor to high blood pressure and vascular disease. National and international organisations recommend that average population salt intakes are reduced to below 6g per day. The objective of this study was to compare the likely costs and benefits of adding a national salt reduction program to existing clinically based disease prevention strategies. We undertook a search for studies reporting the actual or projected costs and/or effects of hypertension management and salt reduction in Australia, summarised the data and compared the costs and effects of the two strategies. We found that hypertension management in Australia costs ~$1 billion per year (~$500 million on drug therapies and $500 million on other costs) and likely prevents approximately 10% of the disease burden attributable to high blood pressure. A national salt reduction strategy to reduce average daily intakes from 9g to 6g in Australia was projected to cost ~$15 million dollars a year, (1–2% of the hypertension program). This would reduce population blood pressure levels by 3–4 mmHg, resulting in a 15–20% reduction in premature vascular disease in the immediate term and a 20–30% reduction in the longer term, due to the cumulative effects of the attenuation of the population rise in blood pressure with age. The costs of the salt reduction program are anticipated to decrease over time. Expansion of hypertension recognition and treatment would also prevent more events but would be less cost effective. In conclusion, there appears to be a strong case for the addition of a nationally coordinated salt reduction program to the existing clinical hypertension control program with substantial health benefits accrued at minimal additional cost.

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

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The endothelin-derived relaxing factors, endothelin-derived hyperpolarizing factor (EDHF) and nitric oxide (NO), can invoke endothelium-dependent hyperpolarisation and relaxation of the vascular smooth muscle. This study investigates the effects of 10-week streptozotocin (STZ)-induced type-1 diabetes on endothelial function in the cremaster artery, a skeletal muscle resistance artery, compared to corresponding control rats. In MOPS (3-(N-morpholino)-propanesulfonic acid)-modified Krebs buffer containing 10 μM indomethacin, endothelium function was assessed using pressure myography by measurement of the internal diameter of isolated segments of cannulated cremaster arteries, maintained at a physiological pressure of
FENOFIBRATE EXAGGERATED ACTH-INDUCED HYPERTENSION IN RATS

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In the present study, we investigated the effect of fenofibrate on glucocorticoid-induced hypertension in rats. Male Sprague-Dawley rats were treated with saline, adrenocorticotropic hormone (ACTH) (0.2 mg/kg/day) or dexamethasone (0.03 mg/rat/day) s.c. for 13 days. Fenofibrate (100 mg/kg/day per rat) was mixed in ground food. Systolic blood pressure (SBP) was measured by tail-cuff method. Aortic superoxide production was measured by lucigenin-enhanced chemiluminescence. Thymus weight was measured as a marker of glucocorticoid activity and plasma NO2 concentrations as a marker of NO production. 20-HETE production was measured by LC/MS/MS. Relative to saline, SBP was increased by ACTH (108.0±1 to 133.4±4 mmHg, P<0.0005) and dexamethasone (109±1 to 140±4 mmHg, P=0.0005). Fenofibrate increased SBP in ACTH-treated rats (to 148±3.3 mmHg (P<0.005) but did not change SBP in dexamethasone-treated rats. Both ACTH and dexamethasone increased renal microsomal 20-HETE formation and thymus weight. ACTH increased aortic superoxide production while dexamethasone decreased plasma NO2 concentrations. Fenofibrate inhibited renal 20-HETE formation in ACTH- and dexamethasone-treated rats but had no effect on thymus weight, plasma NO2 + 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 MICROSCOPY

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Growth restriction in utero as a result of maternal malnutrition has been linked with gender specific decline in cardiac performance during adulthood. Here we examined the effect of growth restriction in rats, due to maternal low protein diet (LPD), on the changes in fibrosis and biochemical composition of the heart in the female offspring in adulthood. Wistar Kyoto (WKY) dams were administered either LPD (6.7% casein) during pregnancy and lactation or normal protein diet (NPD; 20% casein). At 14 weeks of age, hypertension was induced through a 4 week continuous infusion of angiotensin II (ANGII 200ng/kg/min) in female NPD and LPD offspring (N=7) via subcutaneous minipumps while control group received saline. Arterial blood pressure was measured using a tail cuff method. At 18 weeks of age the offspring were perfused fixed and organs collected. The hearts were weighed, sliced and the heart volumes stereologically determined according to the Cavalieri method. Alternate left ventricle (LV) slices from normotensive and hypertensive offspring were used for assessment with Fourier transform infrared (FTIR) imaging micro-spectroscopy. FTIR images were processed using Unsupervised Hierarchical Cluster Analysis. Birth weights of the LPD offspring were significantly lower compared to NPD (6.5±0.3 g and 7.6±0.3 g, respectively), however in adulthood (18 weeks of age) this difference was no longer significant. ANGII infusion resulted in lower body weight in NPD and LPD offspring (P<0.0002). Absolute heart and LV volumes were both significantly lower in LPD offspring. Relative heart and LV volumes were significantly increased by ANGII administration. Perivascular fibrosis and both media to lumen and adventitia to lumen ratios were increased in the NPD hearts only (P<0.009 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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