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IN VIVO CARDIOVASCULAR RESPONSES OF INTERLEUKIN-6 ARE AGE DEPENDENT

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Elevated circulatory levels of interleukin-6 (IL-6) have been linked to the pathogenesis of several cardiovascular disease states. Nevertheless, a paucity of data exists on the in vivo cardiovascular actions of IL-6 itself, and its potential to influence the effects of other vasoactive agents. We tested the hypothesis that cardiovascular responses to elevated circulatory levels of IL-6 are altered in ageing. The effect of a single bolus (2ng.kg.i.v.) of recombinant human IL-6 on femoral mean arterial blood pressure (MAP), heart rate (HR) and tail vein blood flow (BV) was investigated in young (8–10 wk), old (12 mo), and young rats (WKY) treated with 40 mg/kg/day L-NAME for 2 weeks. MAP, HR and BV responses to phenylephrine (PE) and sodium nitroprusside (SNP) were also evaluated (1, 4.2 μg.kg.i.v.) before and after the IL-6 administration. Compared to the saline controls, IL-6 caused time-dependent increases in MAP and heart rate (P<0.05), BV increased (P>0.05) in young rats while no change in MAP or BV were apparent in the aged and the L-NAME treated rats. HR was unchanged in aged rats but increased following L-NAME treatment, suggesting an intact baroreceptor buffering in the young, IL-6 pre-treatment decreased the responsiveness to PE (P<0.05) in young (10–22%) and aged rats (45–52%), an effect that was offset following treatment with L-NAME. In young rats, SNP caused significant decrease in MAP that was unchanged by the elevated circulatory IL-6. In contrast, young rats pre-treated with IL-6 showed a significant increase in HR (11.0±2.4 vs 24.5±5.3 bpm, after IL-6, P<0.05) that was independent of the SNP dose. This effect was absent in the aged rats. In summary, low circulatory levels of IL-6 potentially exert direct cardiovascular effects as well as influencing the responsiveness to other vasomodulatory agents which are age-dependent. Results also suggest age-related structural changes of the cardiovascular system, central autonomic function and endothelial dysfunction as key determinants of IL-6 induced cardiovascular responses.

MEAN ANNUAL COSTS OF ANTHYPERTENSIVE MEDICATIONS AMONG PATIENTS WITH, OR AT RISK OF ATEROTHROMBOSIS - DATA FROM THE REDUCTION OF ATEROTHROMBOSIS FOR CONTINUED HEATH (REACH) REGISTRY

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The relative ease of non-invasive estimation of central aortic pressure has enabled interpretation of age-related changes in the pressure waveform in terms of altered vascular properties affecting wave propagation. In contrast, non-invasive recordings of aortic flow wave patterns are not as common, but limited invasive (electromagnetic) and non-invasive (doppler) recordings indicate ageing changes in the elderly due to altered ventricular function. The aim of this study was to determine changes in aortic flow in subjects in whom central aortic pressure is available, based on known age-related changes in aortic impedance. A parametric model was obtained from studies in the literature by fitting impedance modulus and phase (as a function of frequency) with high order polynomials. Age-related functions were obtained for polynomial coefficients. Regression relations enabled the construction of age-related family of impedance curves, which were then applied to pressure waveforms in individual subjects to determine flow frequency components and to resynthesise flow waves.

Disparities in the pressure waveforms were noted between the different disease groups, as was a difference in the patterns of change between the different age groups. This suggests that the response of aortic flow to age may be different in various arterial disease states. Therefore, non-invasive determination of aortic flow changes from the central aortic pressure is a potential tool with the development of non-invasive central aortic pressure wave age-related model coefficients. Patterns also indicated changes in late systolic flow velocities occurring at age ranges with little change in systolic pressure augmentation. The study showed that the known age-related changes of the ascending aortic impedance can be quantified by polynomials to enable non-invasive determination of aortic flow changes from the central aortic pressure wave.
we examined whether the function of central pathways regulating sympathetic reflexes (chemoreflex induced by 10% O2 and 3% CO2 and responses to stress (airjet and oscillation, each for 10 minutes) is altered in conscious rabbits infused with low dose Ang II (10–30 ng/kg/min) for 5 weeks or by daily stress (airjet) for 1 week. A separate group received both treatments and a control group was included. We also determined the contribution of central sympathetic production in the response to stress using the suprapontine scavenger Tempol. All rabbits were instrumented with an intracerebroventricular (ICV) catheter and an electrode to measure renal sympathetic nerve activity (RSNA). MAP was elevated at the end of the Ang treatment to 92 ± 2 from a baseline of 71 ± 0.2 mmHg (n = 16). Ang had no effect on pressor or for heart rate responses to oscillation or airjet but the RSNA response to hypoxia was greater than in control rabbits. Exposure to chronic stress did not alter MAP or RSNA but the pressor response to oscillation stress was attenuated by 43% (P = 0.03). By contrast the pressor and RSNA response to hypoxia were markedly amplified by chronic stress (P < 0.02). Tempol (400 mg/kg IV) had little effect on the response to oscillation stress in control rabbits but markedly reduced the pressor and RSNA responses to stress in Ang and chronically stressed rabbits. Our results show that chronic treatments with stress or low dose Ang in rabbits not only increase the responsiveness of specific CNS pathways regulating sympathetic nervous system reflexes but also increase their dependence on suprachiasmatic production.

**EARLY INTENSIVE BLOOD PRESSURE LOWERING ENHANCES HAEMATOMA RESOLUTION BUT DOES NOT AFFECT PERIHAEATOMA OEDEMA: THE INTENSIVE BLOOD PRESSURE REDUCTION IN ACUTE CEREBRAL HAEMORRHAGE TRIAL (INTERACT) STUDY**

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The Intensive Blood Pressure Reduction in Acute Cerebral Haemorrhage Trial (INTERACT) has shown that the early lowering of elevated blood pressure (BP) can attenuate haematoma growth at 24 hours after the onset of spontaneous intracerebral haemorrhage (ICH). The objective of the present analysis is to determine the effects of early BP treatment on haematoma and perihaeatomata oedema volumes over 72 hours. INTERACT included 404 patients (mean age 62 years, male 65%, Chinese 95%) with CT-confirmed ICH and elevated systolic BP (>150 and ≤220 mmHg) with the capacity to commence BP lowering treatment within 6 hours of ICH. Patients were randomly assigned to an intensive (target systolic BP 140 mm Hg) or conservative (target systolic BP 180 mm Hg) BP lowering strategy using routine BP available intravenous agents. Digital images of baseline and repeat CT (24 ± 3 and 72 ± 3 hours) were performed using standardized techniques and analyzed centrally. Efficacy measures on available repeat images were relative and absolute changes in haematoma (n=276) and perihaeatomata oedema volumes (n=270) volumes at 24 and 72 hours. Among patients with 3 sequential CT scans, mean (SD) haematoma volumes (ml) were 13.2 (13.0) in the intensive group compared to 12.0 (10.9) in the guideline group. Compared with the guideline group, mean systolic BP was 12.3 mm Hg lower (P<0.0001) in the intensive group in the 1 to 24 hour period post-randomisation. Mean haematoma volumes (ml) at 24 and 72 hours were 13.3 (11.9) and 11.9 (10.5) in the intensive group compared to 15.3 (16.1) and 13.4 (12.8) in the guideline group; differences in mean absolute volumes between randomised at this time points were –3.2% (95% CI –1.0 to –5.4) and –2.7% (95% CI –0.8 to –4.6), respectively (P<0.0001). After controlling for baseline haematoma volume, time to CT, and haematoma location, perihaeatomata oedema volumes (ml) at baseline, 24 and 72 hours, were similar at 9.0 (8.7), 14.5 (13.3) and 18.7 (17.1), and 9.2 (8.5), 16.2 (18.7) and 20.8 (22.8), in the intensive and guideline groups, respectively. Early intensive BP lowering treatment had effects of both attenuating growth and enhancing resolution of the haematoma over 72 hours after the onset of ICH. There were no appreciable effects on perihaeatomata oedema.


**ASSOCIATION OF GLAUCOMA WITH CENTRAL BLOOD PRESSURE WAVEFORM PARAMETERS**

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Background. Glaucoma is theorised as being associated with blood pressure parameters, though studies to date have not been consistent in their findings. This study aimed to investigate peripheral and central blood pressure parameters in definite glaucoma sufferers. Methods. 107 glaucoma subjects were studied, where glaucoma was strictly defined as definite glaucoma with a visual field defect. Brachial systolic/diastolic pressures and radial pressure waveforms were measured and calculated. Bland-Altman plots were constructed for each algorithm, and repeatability calculated. Agreement between different methods was calculated using repeated measures analysis results. Results. The intersecting lines of fit during late diastole / early systole had the highest success rate followed by the phase-slope algorithm (99%; 98% respectively). Repeatability of measurement was highest using the phase-slope algorithm followed by the intersecting lines algorithm (standard deviation of differences 1.86; 2.22 m/s respectively). The intersecting lines algorithm had the best agreement with other pulse transit time algorithms. Conclusions.Whilst agreement between several PWV algorithms was high, no one algorithm was better in all categories. The intersecting lines algorithm was most robust and the phase-slope algorithm showed the greatest repeatability. These findings are important in aPWV measurement, and for reliable and accurate PWV measurement in general.

**HYPERDYNAMIC ANALYSIS OF FLOW DISTURBANCES IN CEREBRAL ANEURYSMS**

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Background. With increased imaging facilities there is a greater rate of detection cerebral aneurysms. This presents a decision problem in terms of the relative risk involved in surgical intervention or the risk of aneurysm growth and rupture. The aim of this study is to simulate blood flow dynamics in a finite volume model (FVM) of vascular malformations with varying geometry of the aneurysm and parent artery. Methods. FVM analysis was conducted on vascular structures representing aneurysms and parent arteries. Aneurysm size, determined as the maximum linear dimension in a single plane, was in the range 6–10 mm; parent artery diameter, 4–8 mm. Simulations were performed with pulsatile and steady pressure and flow (nominal value 0.3 l/min) using normal values of blood density (1.05 g/ml) and viscosity (0.0035 Pa.s). Results were compared in terms of the aspect ratio (AR) of the aneurysm (AR=depth/size). Results. For constant AR, both the flow rate and maximum velocity in the aneurysmal cavity was dependent on the radius of curvature of the parent artery. Variations in AR produced smaller relative changes in haemodynamic flow patterns with constant dimension and configuration of parent artery. Conclusions. Numerical simulation of vascular malformations enables analysis of blood flow in aneurysm and provides information not available with conventional imaging modalities. This study has shown that factors other than aneurysm size, as commonly used for decisions on surgical intervention or treatment, are responsible for disturbed flow dynamics that can increase the risk of rupture.

**CHANGES IN ENDOTHELium-DERIVED HYPERPOLARISATinG FACTor (EdHF) IN RENAL ANEURYSM FROM type-2 DIABETIC RATS**

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This study compared endothelial function in 3rd-order renal arteries from male type-2 diabetic obese Zucker rats (OZR, blood glucose: 13.4 ± 0.6 mmol, n = 22; HDZ, 9.6 ± 0.1%, n = 11) and control lean Zucker rats (LZR, blood glucose: 9.8 ± 0.5 mmol, n = 21; HDZ: 6.1 ± 0.1%, n = 11) due to blood flow to the eye, especially during diastole, in a positive pressure (intracocular) environment. Further work is required to associate these systemic parameters with local ocular blood flow conditions.
at 25 weeks of age. Endothelial function was assessed in a Mulfaryn-Halpern wire myograph in the presence of 10 μM indomethacin. Relaxations to the endothelium-dependent dilator ACh and the endothelium-independent dilator sodium nitroprusside were not different in arteries from diabetic and control rats. Blockade of the nitric oxide (NO) pathway using nitro-l-arginine methyl ester (100 μM) and ODQ (1 μM) significantly reduced to ACh (7:10−5, n=5). Furthermore, the residual relaxation was not significantly different when compared to LBR (Emax reduced from 86:1% to 82:1%, n=20). The residual relaxation was abolished in both groups of rats (n=5–6) by inhibition of large-, intermediate- and small-conductance Ca2+–activated K+ channels using charybdotoxin (0.1 μM) and apamin (1 μM); respectively. The 20-HETE response was not different between the 100 μM indomethacin and its inactive isomer (10 μM) nor between the 100 μM indomethacin and the inactive isomer. The 20-HETE response was not different in the arteries from type 2 diabetic OZR, there is an alteration in the balance of NO and EDHF components of the response. Endothelial NOS is increased in the OZR and there also appears to be an upregulation of connexin 43-associated gap junction activity in the OZR.

20-HYDROXY EICOSATRIENIC ACID SYNTHESIS IN HUMAN PLATELETS AND NEUTROPHILS AND THE ROLE OF ANGIOTENSIN II AND ENDOTHELIN-1

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Metabolism of arachidonic acid through the cytochrome P450 pathway leads to formation of 20-hydroxy eicosatrienic acid (20-HETE) that is a vasconstrictor in many vessel beds. 20-HETE acts as a second messenger for vasconstrictor actions of Angiotensin II (AI) and Endothelin (ET-1) in renal and mesenteric beds in vivo. We have previously reported that calyculin A significantly reduced the NAME/OO50-resistant response in OZR (Emax reduced from 29±1% to 44±2%). The residual relaxation was not significantly different in arteries from type 2 diabetic OZR, there is an alteration in the balance of NO and EDHF components of the response. Endothelial NOS is increased in the OZR and there also appears to be an upregulation of connexin 43-associated gap junction activity in the OZR.

201 IMMUNOHISTOCHEMICAL DETECTION OF ADVANCED GLYCATION END-PRODUCTS (AGEs) IN DIABETIC TISSUES: RELEVANCE TO ANTIGEN RETRIEVAL TECHNIQUES

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In hyperglycaemic state found in diabetics, an accelerated non-enzymatic glycation and oxidation of proteins and lipids occurs. This leads to the formation of advanced glycation end-products (AGEs). These products can accumulate in the plasma and tissues of diabetic subjects and may contribute to the development and progression of vascular complications associated with the disease. The method of one to analyse their burden is immunohistochemical detection paraffin blocks proceeded by AGEs epitopes retrieval. Antigen retrieval (AR) is a technique that re-exposes epitopes in formalin fixed, paraffin embedded tissue sections and makes them detectable by immunohistochemistry. The aim of this study was to determine optimal retrieval techniques to detect AGEs in paraffin embedded tissues samples. The standard immunohistochemical technique was applied to aortic punch biopsy blocks from control and diabetic patients. AGEs presence was detected using three different antibodies with four AR protocols, involving different pH and temperatures. The three antibodies were: polyclonal anti-RAGE (1:800 dilution), monoclonal anti-AGE (6D12, 4 μg/ml) and monoclonal anti-CEL (4F5, 10.4 μg/ml). We used three antigen retrieval techniques, involving different pH and temperatures. The three antibodies were: polyclonal anti-RAGE (1:8000 dilution), monoclonal anti-AGE (4D12, 4 μg/ml) and monoclonal anti-CEL (4F5, 10.4 μg/ml) antibodies. The AR solutions used were DAKO citrate buffer (pH=6.6, at either 60 or 100°C), combined citric acid (10 mM)+ EDTA (2 mM) (pH=7, both at 60°C), boric acid (0.2 M) (pH=6, at 60°C, and Proteinase K (3 min, room temperature). Anti-AGEs and anti-CEL antibodies showed reliable, reproducible results when combined with enzyme-based AR, whereas non-enzyme based AR techniques produced inconsistent results. Results involving anti-RAGE antibody showed positive tissue staining irrespective of type of AR technique. The intensity of the staining was also markedly different between various solutions and experimental conditions by being more intense when Proteinase K was used. In conclusion, we provided evidence that under the current experimental conditions, the enzyme based AR namely Proteinase K (3 min) is the most consistent and reliable technique. We support a notion that in diabetic tissues false positive immunohistochemical staining may be obtained due to possible heat generated AGEs. Using Proteinase K may provide an alternative AR technique when diabetic tissues are involved.

AUGMENTED MYOCARDIAL PI3-K MEDIATED SIGNALLING IN THE HYPOXIC PRECEDES ADULT PATHOLOGICAL HYPERTROPHY IN THE HYPERTROPHIC HEART (HRH)

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Hypertrophy of the heart represents a significant cardiovascular risk, independent of blood pressure. There is growing appreciation that adult cardiovascular disease states may be
THE EFFECTS OF MODERATE PRETERM BIRTH IN LAMBS ON CARDIAC DEVELOPMENT AND BLOOD PRESSURE

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There is emerging epidemiological evidence linking preterm birth with increased blood pressure and adverse health outcomes later in life. As being born prematurely abruptly changes whole body haemodynamics, we postulated that preterm birth would have an adverse impact on cardiac structure, cardiomyocyte endowment and arterial pressure. We have examined this hypothesis using moderate preterm birth in sheep, a species in which cardiomyocyte maturation closely resembles that in humans. Preterm birth (n = 7) was induced at 133 days of gestation and term lambs (n = 8) born at 147 days were used as controls. Arterial pressure was measured via a femoral arterial catheter at 4 weeks and 8 weeks post term equivalent age (PTEA). Lambs were euthanized at 9 weeks PTEA. Using unbiased stereology the total number of cardiomyocytes within the lamb heart was determined. Additionally, cardiomyocyte proliferation and the level of interstitial fibrosis were examined. At 9 weeks PTEA, there was no difference between term and preterm lambs in cardiomyocyte number in the right ventricle in the left ventricle plus septum. There was, however, a significantly greater degree of interstitial fibrosis in preterm lambs compared to controls (0.74% ± 0.61 vs. 4.8% ± 1.45; p < 0.001). There were no significant differences between groups in arterial pressure at 4 and 8 weeks PTEA; however, at 4 weeks PTEA heart rate in the preterm lambs was greater than in controls, but this was not present at 8 weeks. In conclusion, moderately preterm birth leads to an increase in collagen deposition in the 9 week PTEA lamb heart, which may adversely impact on cardiac contractility.

VASODEPRESSOR EFFECT OF COMPOUND 21 IS VIA STIMULATION OF AT2R IN CONSCIOUS SHR

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Angiotensin II (Ang II), the main effector of the renin-angiotensin system (RAS), plays an important role in blood pressure regulation by influencing vascular tone, structure, fluid and electrolyte balance. Most of the established cardiovascular functions of Ang II, including vasoconstriction, are mediated via Ang II type 1 receptor (AT1R). Ang II type 2 receptor (AT2R) stimulation causes a more subtle vasodilator response, which partially counteracts AT1R-mediated vasoconstriction. However, there are many confounding issues in regards to functional manifestation of AT2R, such as paucity of agonists and antagonists, and lack of dose-response analysis in vivo. Moreover, collective in vivo data, suggests that AT2R function is only unmasked during AT1R blockade. Recently, Wan et al., 2004, reported the first non-selective, selective AT2R antagonist, Compound 21, with a Kd value of 0.4 nM for the AT2R and a Kd > 10 µM for the AT1R. Therefore, the aim of this study was to investigate functional cardiovascular effects of Compound 21, and to determine whether or not these effects are AT2R-mediated. Under anaesthesia, adult (17 weeks) male spontaneously hypertensive rats (SHR) were anaesthetised with catheters for direct blood pressure measurement (carotid artery) and intraavenous drug administration (jugular vein). Following 24 hours recovery, rats received drug combinations in a randomised fashion over a 5-day protocol. In initial experiments, Compound 21 was administered alone, at doses ranging from 10ng/kg/min to 1000ng/kg/min for 4 hours. In these experiments, Compound 21 did not decrease blood pressure (BP) in conscious SHR. In analogous experiment in separate animals, Compound 21 was also tested alone (50ng/kg/min), or in combination with the AT1 antagonist, candesartan (0.01 or 0.1 mg/kg). Compound 21 evoked a significant depressor response in adult SHR (~30 mmHg) only during AT1R blockade. Moreover, Compound 21 inhibited AT2R antagonism and fish oil supplementation favourably alter cardiovascular function in patients with coronary disease and post myocardial infarction. However, the effect of lifestyle interventions on heart and vascular function of disease-free, overweight young women appears to be undetermined. Therefore, the effects of 12 weeks of high intensity intermittent exercise training combined with a Mediterranean-style eating plan and fish oil supplement (1.8 g per day) on resting cardiovascular function was examined in 32 overweight (BMI 27.7±6.0 kg/m²) non-smoked premenopausal young women (22.0±6.6 years). Peak oxygen uptake was assessed using open-circuit spirometry (True Max 2400, ParvoMedics) and body composition was measured using dual energy X-ray absorptiometry (DPX-L, Lunar). Basal blood flow was assessed using plethysmography, whereas parasymphatic influence of the heart was assessed through spectral analysis of the inter beat interval. Arterial baroreceptor sensitivity was determined from spontaneous fluctuation of blood pressure and RR interval using sequence method. Augmentation index, a measure of arterial stiffness, was also assessed through applanation tonometry. Results indicate that compared to the control condition, the 12-week intervention significantly reduced body fat (p < 0.001) and improved aerobic fitness (p < 0.001). Resting heart rate, rate pressure product, and arterial stiffness were also significantly reduced (p < 0.05), whereas high frequency power (p < 0.01) of resting heart period variables, and arterial baroreflex sensitivity were significantly increased (p < 0.05). In conclusion, a lifestyle intervention that included intermittent sprint training, a Mediterranean-style eating plan, and a fish oil supplement significantly enhanced the carotid, vascular, and autonomic function of young, overweight women.

THE EFFECTS OF HIGH INTENSITY INTERMITTENT EXERCISE TRAINING COMBINED WITH A MEDITERRANEAN-STYLE EATING PLAN AND FISH OIL SUPPLEMENT ON CARDIOVASCULAR FUNCTION OF OVERWEIGHT WOMEN

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Obesity/overweight has been reported to be associated with disturbances in cardiovascular function. There is evidence that lifestyle interventions involving exercise, healthy eating, and fish oil supplementation favourably alter cardiovascular aberrations in patients with coronary disease and post myocardial infarction. However, the effect of lifestyle interventions on heart and vascular function of disease-free, overweight young women appears to be undetermined. Therefore, the effects of 12 weeks of high intensity intermittent exercise training combined with a Mediterranean-style eating plan and fish oil supplement (1.8 g per day) on resting cardiovascular function was examined in 32 overweight (BMI 27.7±6.0 kg/m²) non-smoked premenopausal young women (22.0±6.6 years). Peak oxygen uptake was assessed using open-circuit spirometry (True Max 2400, ParvoMedics) and body composition was measured using dual energy X-ray absorptiometry (DPX-L, Lunar). Basal blood flow was assessed using plethysmography, whereas parasymphatic influence of the heart was assessed through spectral analysis of the inter beat interval. Arterial baroreceptor sensitivity was determined from spontaneous fluctuation of blood pressure and RR interval using sequence method. Augmentation index, a measure of arterial stiffness, was also assessed through applanation tonometry. Results indicate that compared to the control condition, the 12-week intervention significantly reduced body fat (p < 0.001) and improved aerobic fitness (p < 0.001). Resting heart rate, rate pressure product, and arterial stiffness were also significantly reduced (p < 0.05), whereas high frequency power (p < 0.01) of resting heart period variables, and arterial baroreflex sensitivity were significantly increased (p < 0.05). In conclusion, a lifestyle intervention that included intermittent sprint training, a Mediterranean-style eating plan, and a fish oil supplement significantly enhanced the carotid, vascular, and autonomic function of young, overweight women.

THE EFFECTS OF 4 WEEKS OF MODERATE-INTENSITY EXERCISE ON BARORECEPTOR SENSITIVITY AND ARTERIAL STIFFNESS IN NORMOTENSIVE YOUNG OFFSPRING OF HYPERTENSIVES

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It is well-established that individuals with a family history of hypertension exhibit abnormal baroreceptor sensitivity and arterial stiffness. The purpose of this study was to examine whether a 4-week moderate-intensity cycle exercise intervention could improve cardiovascular function, particularly cardiopulmonary baroreceptor (CPBR) sensitivity and arterial stiffness of individuals with a family history of hypertension. Young individuals, aged 18–27 yrs, with a family history of hypertension (N = 14) participated in this study. Subjects were randomly assigned into exercise (n = 9) and control (n = 5) groups. In Session 1, all subjects underwent a maximal oxygen uptake (VO2max) test. In Session 2, subject’s cardiovascular function was assessed. CPBR sensitivity was calculated by forearm vascular resistance (FVR) change divided by stroke volume change during ~20 mmHg of lower body negative pressure. Arterial stiffness was measured using applanation tonometry by obtaining augmentation index. FVR was calculated by forearm blood flow (FBF) divided by mean arterial pressure. FVR was measured by venous occlusion plethysmography and venous occlusion technique. Blood pressure was monitored continuously using a beat-by-beat tonometry blood pressure sensor (Jentzol, Colin Medical). Stroke volume was assessed non-invasively using impedance cardiography. In Session 3, the exercise group performed 30 minutes of cycling at an intensity of 65% of their VO2max, three times a week for 4 weeks. Control subjects were asked to maintain their normal levels of physical activity. In Session 4, all subjects’ cardiovascular function was reassessed as in Session 2. Results showed that CPBR (Fig. 1) and arterial stiffness (Fig. 2) improved following 4 weeks of moderate intensity of cycle exercise (p < 0.05). The major finding was that the 4 weeks of moderate intensity cycle exercise normalized CPBR and arterial stiffness in young individuals with a family history of hypertension.

ADENYLYL CYCLASE ISOFORM mRNA EXPRESSION WITHIN THE ROSTRAL VENTROLATERAL MEDULLA IN SPONTANEOUSLY HYPERTENSIVE RATS

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Basal levels of sympathetic nerve activity (SNA) controlling vasomotor tone are maintained by neurons in the rostral ventrolateral medulla (RVLM). In hypertension, SNA is elevated, however the cause for this elevation is yet to be described. Adenylate Cyclases (ACs) are important
instigators of intracellular signalling cascades which lead to changes in cell excitability. There are ten known isoforms of ACs, grouped according to regulatory properties and amino acid sequence homology. We compared relative gene expression of the ten AC isoforms in the RVM of a genetically hypertensive model, the Spontaneously Hypertensive Rat (SHR; n=5) and its related normotensive control, the Wistar Kyoto Rat (WKY; n=5). After tail-cuff plethysmography to validate blood pressure phenotypes, animals were perfused and RVMs were dissected, followed by RQLM tissue extraction and RNA isolation. Reverse transcription was carried out on 300 ng of total RNA. PCR was performed for the ten known AC isoforms to confirm their presence or absence within RLM. Real-time PCR was then conducted on five isoforms- ACs 2, 3, 6, 9 and 10 to monitor the level of gene expression between phenotypes. The Pfaffl method of analysis was used to analyse the real-time PCR data, as seen in the table below expressed as both a ratio and percentage. These differences however were not significant when analysed with a Relative Expression Software Tool (P<0.05). In light of this, there appears to be no disparity between SHR and WKY in terms of AC isoforms. However, no real-time PCR analysis of the remaining five isoforms is required to support these findings.

AC Isoform | Expression Ratio (SHR: WKY) | Percentage Increase/ Decrease in SHR
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2 | 1.103 | 10% increase
3 | 0.882 | 12% decrease
6 | 1.227 | 23% increase
9 | 1.230 | 23% increase
10 | 0.907 | 10% decrease

## BLOOD PRESSURE AND THE \( \beta \)-SUBUNIT OF THE EPITHELIAL SODIUM CHANNEL

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The epithelial sodium channel (ENaC) is involved in the long- and short-term regulation of blood pressure (BP) and has been implicated in Mendelian BP diseases Liddle’s syndrome and primary hyper-reninemia. The specific role of each subunit in BP regulation is unclear and needs to be defined. We used an array with a 5303bp median probe to detect alterations in DNA copy number between the SHR and the non-hypertensive WKY model from which the SHR was genetically derived. As a preliminary association analysis of SCNN1B, we performed experiments to determine whether CNVs exist between the SHR and WKY. SCNN1B, is located near or within blood pressure QTLs, more often than would be expected by chance, located in areas where previous QTLs for cardiovascular risk factors reside. CNVs were detected on various rat chromosomes and varied in size from 27 kb to 190 Kb. Interestingly, most of these variations were located in the region of chromosome 16 linked to BP by a number of independent studies, including the Victorian Family Heart Study (VFHS). We have previously reported association of the \( \gamma \)-ENaC gene and BP; here we present our findings for the \( \gamma \)-ENaC gene, SCNN1B, and BP. We performed a preliminary association analysis of SCNN1B on data from 8890 hypertensive and 8888 normotensive subjects who were genotyped using an extreme phenotyping approach, utilising unrelated subjects from the upper and lower deciles of the SBP distribution. This identified four SNPs with some evidence of association to SBP after adjusting for age, sex and BMI (rs1004749 P=0.006; rs2380345 P=0.0009; rs2380346 P=0.0015; rs2747896 P=0.08). To follow up these findings, we genotyped the four SNPs previously associated in the VFHS in 1971 relatives from 68 large Utah pedigrees selected for high risk of cardiovascular disease who had available BP and DNA. Generalised estimating equations were used to test for association of SNPs in SCNN1B and BP while controlling for related observations in families. After adjusting for the covariates age, sex and weight index, we detected significant association for SNP rs2380345 with SBP and DBP (p=0.03) at baseline. In conclusion, the SCNN1SNP rs2380345 appears to be associated with BP in the WKY and Utah pedigrees.

## JOINT EFFECTS OF BLOOD PRESSURE LOWERING AND GLUCOSE CONTROL IN THE ADVANCE TRIAL


Background: The ADVANCE trial has reported the separate benefits of blood pressure (BP) lowering with the fixed combination of perindopril and indapamide and of intensive glucose control with a glitazide MR-based regimen on a range of cardiovascular and renal events in patients with type 2 diabetes. The extent to which the effects of these interventions may be independent and additive remains uncertain.

Methods: The hazard ratios and the total number of events occurring during the follow-up period, averaging 4.5 years to the end of the BP lowering comparison, were analysed using Cox models with interaction terms in the four groups randomised to perindopril-indapamide alone, to glitazide MR-based regimen alone, to both together or to neither, with a total of 11,140 participants, evenly distributed across all 4 groups. The outcomes examined were pre-specified ADVANCE endpoints.

Results: There was no interaction between the effects of the routine BP lowering treatment and the intensive glucose control regimen for the pre-specified primary or secondary clinical outcomes (p>0.1 for all), suggesting that the separate effects of these 2 interventions were independent and additive. For outcomes where both treatments caused separately significant risk reductions, these were fully additive as seen with new or worsening nephropathy which was reduced by one third (p=0.005). When only one treatment had a significant effect, the second intervention did not undo that effect, and in some cases augmented it, as was seen with all-cause mortality, which was reduced by 14% by the BP lowering treatment (p=0.025) but by 18% for the group receiving both active treatments (p=0.04).

Conclusions: The separate effects of routine BP lowering with the fixed combination of perindopril and indapamide and of intensive glucose control with the glitazide MR-based regimen were independent for all pre-specified primary and secondary outcomes. The joint effects yielded substantial benefit reductions of around one third in nephropathy and close to one fifth in total cardiovascular death. Multivariable risk ratio estimates including BP lowering and intensive glucose control are indicated for all patients with type 2 diabetes.

## SOMATIC STIMULATION REVEALS UNIQUE RESPONSE PATTERNS IN DIFFERENT SYMPATHETIC NERVES IN THE RAT

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Results of many studies support a theory that presympathetic vasomotor neurons in the rostral ventrolateral medulla (RVM) provide tissue-specific control. The aim of this study was to identify functionally distinct effenter projections of RVM in presympathetic neurons by axon conduction velocity and catecholamine phenotype. We simultaneously recorded the sympathetic outflow response of multiple, sympathetic nerves – cervical, renal and splancnic – to sciatic nerve stimulation (single 0.2 ms pulse, 50 sweeps at 0.5 Hz, 1–50 V), in urethane-anaesthetised (1.3 g/kg i.p.), paralysed, vagotomised and artificially ventilated Sprague Dawley rats (n=14). Furthermore, splancnic somato-sympathetic responses (SSR) was examined in rats treated with intrasplanchnic anti-dopamine-beta-hydroxylase-saporin (24 mg/side, n=8), a neurotoxin that depleted 60–80% of catecholamine neurons in the RVM compared to IgG-saporin control (n=10). We observed qualitatively different SSR responses in all nerves with increasing sympathetic tone which spared neurons that appear to have higher conduction velocities. The unique temporal features of each SSR reveal distinct spinal projections of presympathetic RVM neurones.

## WHOLE GENOME SURVEY OF COPY NUMBER VARIATION BETWEEN THE SPONTANEOUSLY HYPERTENSIVE RAT AND THE WISTAR-KYOTO RAT

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The spontaneously hypertensive rat (SHR) is one of the most widely used genetic models for hypertension. Copy number variation (CNV) is defined as a DNA segments that is >1 kb and is present at a variable copy number in comparison with a reference genome. CNVs are increasingly recognized as a source of inter-individual differences in disease and may affect gene expression or function contributing to heritable differences for blood pressure. We performed experiments to determine whether CNVs exist between the SHR and the normotensive Wistar-Kyoto (WKY) which may possibly play a role in the disease progression in the SHR. We performed a 220,000 genographic hybridization (CGH) using a whole-genome array with a 5303bp median probe to detect alterations in DNA copy number between the SHR model and the non-hypertensive WKY model from which the SHR was genetically derived. The significance of association to SBP after adjusting for age, sex and BMI (rs2380345 P=0.08; rs2380346 P=0.0015; rs2747896 P=0.08). To follow up these findings, we genotyped detected on autosomes 1, 3, 4, 6, 7, 10, 14 and 17. CNVs detected in this study seem to be located near or within blood pressure QTLs, more often than would be expected by chance.
which supports the hypothesis that CNVs are causally linked. Importantly, many of the CNVs contain known genes and thus may underlie both gene expression and phenotypic variation between the rat models. Further studies and finer tilting arrays are warranted.

026

DOES THE REACTIVITY OF THE SYMPATHETIC NERVOUS SYSTEM CONTRIBUTE TO THE MORNING SURGE IN BLOOD PRESSURE?

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The early morning is known to be associated with increased cardiovascular risk. Hypertensive individuals, who have a greater risk of cardiovascular events, also have an increased amplitude, faster rate and more frequent rise of BP during the morning. Although the mechanisms underlying this relationship remain unclear, it is possible that an exaggerated response to arousal is a important factor. We therefore determined whether the reactivity of the sympathetic nervous system (SNS) is related to the morning surge in blood pressure. Ambulatory blood pressure monitoring (ABPM) was obtained from 102 normotensive men and the amplitude and frequency of rise (RoR) of morning mean arterial pressure were determined mathematically. In addition, we determined a measure of the effective power of the morning surge in mean arterial pressure (BPPOWER), derived by the product of the amplitude and RoR. The reactivity of the SNS to an average stimulus was assessed on a separate day by microneurographic recording of multisynaptic, postganglionic muscle sympathetic nerve activity (MSNA), measured from the peroneal nerve at the fibular head. Blood pressure and electrocardiogram were measured concurrently at rest, during a cold pressor test which involved immersing the hand in ice water for 2 minutes, and at recovery. We examined 33 subjects (14 males / 19 females ) with average age 40±6.4 years (range 16 – 83), BMI 25.9±0.7 and 24% of whom were taking antihypertensive therapy. The cold pressor test increased MSNA (P<0.001) and mean arterial pressure by 24±0.2±4 mmHg (P<0.001). MSNA was adjusted for age and BMI, and subjects were divided into tertiles by RoR and BPPOWER. RoR and BPPOWER were positively related to the increase in total MSNA (r = 0.4, P<0.02) and MSNA amplitude (r = 0.5, P<0.01) observed during the cold pressor test but were not related to the increase in MSNA frequency (r = 0.03). In conclusion, these results suggest that the CNS mechanisms influencing the increase in sympathetic burst amplitude during arousal may also be fundamental in determining the rate of blood pressure rise during the morning period.

027

FEATURES OF FLOWING OF METABOLIC SYNDROME ASSOCIATED WITH HYPERTENSION

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As a result of present lifestyle (abundance of physical activity, increased intake of calories, etc.), number of persons with impaired glucose tolerance, cardiometabolic diseases, diabetes mellitus and obesity is elevated. According the estimation, worldwide 20–25% population suffers from metabolic syndrome (MS). In this population, all cause mortality is two times higher and risk of cardiovascular event is three times higher than in persons without metabolic syndrome. Prevalence of MS is growing to an epidemic in the developed countries. The subject of this research was 73 persons with metabolic syndrome (MS) by age 33–57 years, 38 of them were patients with arterial hypertension (AH). Patients underwent the next procedures: determination of anthropometric indexes (body mass index (BMI), circumference of waist), determination of concentration of glucose and lipidemia, determination of anthropometric indexes (body mass index (BMI), circumference of waist (CW), determination of concentration of glucose and lipidemia. The cold pressor test increased MSNA (P<0.05) and mean arterial pressure by 24.0±2.2 mmHg (P<0.001). MSNA was adjusted for age and BMI, and subjects were divided into tertiles by RoR and BPPOWER. RoR and BPPOWER were positively related to the increase in total MSNA (r = 0.4, P<0.02) and MSNA amplitude (r = 0.5, P<0.01) observed during the cold pressor test but were not related to the increase in MSNA frequency (r = 0.03). In conclusion, these results suggest that the CNS mechanisms influencing the increase in sympathetic burst amplitude during arousal may also be fundamental in determining the rate of blood pressure rise during the morning period.

028

EXAGGERATED BLOOD PRESSURE AND HEART RATE CIRCADIAN RHYTHMS INDUCE ACTIVATION OF HYPOTHALAMIC NEURONS IN GENETICALLY HYPERTENSIVE MICE

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

We have previously shown that the high blood pressure (BP/J2) strain of “Schlager” mice also have greater arterial associated rises in blood pressure compared with normotensive mice (BP/J3). However, it is unclear whether this is due to a neurogenic mechanism or to peripheral vascular and cardiac hypertrophy. Therefore, we examined whether acute averse stress activates hypothalamic brain regions that may contribute to the hypertension observed in these BP/J2 mice. BP/J2 and BP/J3 mice were implanted with telemetry measure mean arterial pressure (MAP), heart rate (HR) and locomotor activity. Following recovery, CV variables were recorded at rest and throughout a 1 hr period when a high arousing state (stress) was induced by placing a mouse in a cage previously occupied by a different male mouse (cage swap). Animals were then perfused and neuronal activation was detected using c-Fos immunohistochemistry. Before stress exposure, greater resting MAP was recorded in BP/J2 mice (102±2:2 mmHg; n=5) than in BP/J3 mice (86±1:1 mmHg; n=7). HR levels were also elevated in BP/J2 mice (348±1:11 bpm) compared with BP/J3 mice (338±0:5: bpm). During cage swap stress MAP increased more in BP/J2 mice compared to BP/J3 mice (4±2 vs ±1:1 mmHg, P<0.001), as did locomotor activity (±6:3±0:2 vs ±2.6±0:2 units, P<0.001). By contrast HR increased less in BP/J2 mice (±275:13 vs ±350±15 bpm, P<0.001). Following exposure to acute stress, neuronal activation (c-Fos expression) was 33% greater in the paraventricular nucleus of the hypothalamus (P<0.05) and 27% greater in the dorsomedial hypothalamus (P=0.001) in BP/J2 mice (n=9) compared to BP/J3 mice (n=9). Our findings show that a relatively “natural” arousal response induces a markedly greater activation of hypothalamic regions that are known to be important for regulating cardiovascular autonomic function. The associated greater pressure response to stress suggests that this may be a major underlying central mechanism contributing to the hypertension.

030

THE ROLE OF AT1A RECEPTORS IN CARDIOVASCULAR REACTIVITY TO ACUTE AVERSE STRESS

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Recently, we have shown that pharmacological inhibition of angiotensin AT1A receptors in the dorsomedial hypothalamus (DMH) attenuates cardiovascular (CV) stress response. In the present study we determined whether reduced CV reactivity in AT1A knockout (AT1A–/–) mice relates to attenuated neuronal responsiveness to stress. AT1A–/– and AT1A+/+ mice were implanted with telemetry devices to measure mean arterial pressure (MAP), heart rate (HR) and locomotor activity. Following recovery, CV responses were recorded at rest and during a 1 hour period when a high arousal state was induced by placing a mouse in a cage previously occupied by a different male mouse (cage swap). Neuronal activation was detected using c-Fos immunohistochemistry. Before stress exposure, greater MAP was recorded in AT1A–/– mice (112±2 mmHg; n=10), whereas HR levels were not different between groups. Cage swap increased MAP by +24±2 mmHg in AT1A+/+ mice and by +17±2 mmHg in AT1A–/– mice, as did HR (±203:9±18 vs ±129:9 bpm; P=0.001). This smaller HR response may be due to the failure of stress to inhibit baroreceptor reflexes in AT1A–/– mice. Likewise, locomotor activity was also less in AT1A–/– mice. Cage swap stress neuronal activation in the bed nucleus of the stria terminalis (P<0.001), paraventricular nucleus, central nucleus of the amygdala, rostral ventrolateral medulla (VLM) (P<0.01), DMH and raphe pallidus nucleus (P<0.05). Thus, the attenuated CV and behavioral responses suggest that primary differences between groups may relate to lesser activation at the limbic level (amygdala) where the primary emotional reaction to stress is formed. This may lead to lesser activation at the hypothalamic autonomic areas and also motor regions. We also observed greater activation in the caudal VLM (P<0.01) and nucleus of the solitary tract (P<0.005) in AT1A+/+ mice compared with AT1A–/– mice which may reflect lesser baroreflex inhibition induced by stress in AT1A–/– mice. These studies suggest that central AT1A receptors are likely involved in the emotional and autonomic reactions to acute averse stress.

031

IS THERE A THRESHOLD EFFECT OF FLAVANOL RICH COCA ON BLOOD PRESSURE?

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The effect of cocoa on BP is controversial. Although flavanol rich cocoa can improve flow mediated dilatation, evidence for a sustained effect on BP is lacking, yet small amounts of dark
chocolate with flavanol intakes as low as 30 mg/day reportedly lower BP. We used 24 hr ambulatory BP (ABP) in individuals with borderline/high BP to test for dose-related antihypertensive effects of flavanol-rich cocoa. Men and postmenopausal women with clinic BP >130/85 and <160/100 mmHg were randomised to consume cocoa beverages (Mars Inc) containing 33, 372, 712 or 1052 mg/d flavanols for 6 weeks (n=14, 12, 13 and 13 respectively) in a double-blind, parallel comparison. Seated clinic BP and 24 hr ABP were measured at 0, 3 and 6 weeks. Changes over time were tested by repeated measures ANCOVA with baseline BP as a covariate. Change from baseline to weeks 3 and 6 were tested with a nested plot ANOVA. There were no significant effects of cocoa on clinic BP. However, there was a dose x time interaction for 24-hr MAP (P=0.047). The nested change from baseline analysis revealed a significant dose effect for 24-hr SBP (P=0.001), DBP (P=0.002) and MAP (P=0.0004, see figure). The 1052 mg dose reduced 24 hr SBP/DBP by 5/3 mmHg; all other doses were ineffective. There appears to be a threshold above 700 mg/d for the antihypertensive effect of cocoa flavanols although this may differ when consumed in dark chocolate.

**Change in 24 hr MAP (mmHg)**

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<th>-1.5</th>
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**302**

**STRESS’ IS ASSOCIATED WITH SYMPATHETIC ACTIVITY IN PEOPLE WITH THE METABOLIC SYNDROME**

T Dawood, EA Lambert, N Stranzicky, M McGarrie, GW Lambert, Human Neurotransmitters Laboratory, Baker IDI Heart and Diabetes Institute, Melbourne

Stress pathways, including the sympathetic nervous system (SNS), have been demonstrated to be activated and involved in generating metabolic abnormalities that characterise the metabolic syndrome. In this study we aimed to determine whether cortisol level and depression contributed to the sympathetic activation evident in people with the metabolic syndrome. Forty-seven untreated subjects, meeting criteria for the metabolic syndrome according to the International Diabetes Federation (2005) guidelines, were recruited. Sympathetic activity was measured using muscle sympathetic nerve activity (MSNA) of the peroneal nerve. Blood pressure was measured using radial arterial tonometry and heart rate was determined using a lead III ECG recording. Anxiety and depression levels were assessed with the Trait section of Spielberger’s State and Trait Anxiety Inventory and Beck Depression Inventory II (BDI-II, respectively). There was a significant positive correlation between BDI-II and MSNA scores, r=0.507, P<0.0001. In people with the metabolic syndrome, higher BDI-II scores were significantly associated with higher MSNA compared with lower scores, 43.2±2 vs 34.3±3 bursts/min (mean±se; P=0.02), respectively. Similarly, higher anxiety scores were associated with higher MSNA, 43.2±2 vs 33.3±3 bursts/min (mean±se; P<0.007). Those subjects with higher depression and anxiety scores also exhibited a worse “metabolic profile”: higher anxiety was associated with elevated triglycerides, 1.85 (1.50–2.05) mmol/L vs 1.45 (1.10–1.70) mmol/L (Median [25–75 percentile]; P=0.018), and higher BDI-II scores were associated with increased cholesterol levels, 6.2±0.2 mmol/L vs 5.6±0.2 mmol/L (mean±se; P<0.05). Furthermore, higher BDI-II scores tended to be associated with insulin area under the curve, 11484 (9445–12969) units vs 9084 (7087–10562) units [Median [25–75 percentile]; P=0.058]. Age, gender and blood pressure were comparable in each group. Our data indicate that people with the metabolic syndrome with higher depression or trait anxiety scores exhibit SNS activity that is higher than that of control individuals. Further research is needed to determine whether higher sympathetic activity can be causally linked to the development of the metabolic syndrome.

**303**

**GENDER INFLUENCES CEREBRAL VASCULAR RESPONSES TO ANGIOTENSIN II-ROLES OF NOX2 DERIVED REACTIVE OXYGEN SPECIES AND RHO-KINASE**

TM De Silva, BRs Broughton, GR Drummond, CG Sobey, AA Miller, JA Ellis1,2, A Lamantia 1, KJ Scurrah 1,3, C Nichols 4, SB Harrap 1, MCA from both genders. Ang II elicted smaller contractions of MCA from female WT mice than from male WT mice (P<0.01), and inhibited cortical Ang II activity (measured by quenched fluorescent substrate assay) were reduced (P<0.05 and P<0.001 respectively). Real-time PCR demonstrated that STNx rats had increased medullary mas receptor mRNA (P<0.001), STNx rats had increased cortical ACE binding (P<0.001) and decreased medullary receptor mRNA (P<0.05). These data suggest that dysregulation of ACE2 may contribute to the renal pathology evident following STNx, and suggest that strategies that increase ACE2 and/or its product Ang (1–7) may be useful in the treatment of renal disease.

**304**

**ROLE FOR RENAL ANGIOTENSIN CONVERTING ENZYME 2 IN THE ADAPTATION TO NEPHRON LOSS FOLLOWING SUBTOTAL NPHRECTOMY IN THE RAT**

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The mechanisms of chronic kidney disease progression remain to be fully elucidated. Angiotensin converting enzyme (ACE) 2 is expressed in the kidney, and is implicated in renal pathology secondary to diabetes and hypertension. ACE 2 catalyzes the cleavage of Angiotensin (Ang) II to Ang (1–7), a peptide with anti-fibrotic actions mediated via the Ang (1–7) or mas receptor. This study assessed the effect of experimental renal ablation on renal ACE2, and investigated the effect of ACE inhibition. Sprague-Dawley rats had sham (control) or subtotal nephrectomy (STNx). STNx rats received either ACE inhibitor (ACEI), 1 mg/kg/day (N=12) or vehicle-N (9) daily orally for 10 days after surgery. STNx rats had polyuria (P<0.001), proteinuria (P<0.01), hypertension (P<0.01), and kidney hypertrophy (P<0.001). In the remnant kidney, autoradiography demonstrated an increase in cortical ACE binding (P<0.005), whilst cortical and medullary ACE2 activity (measured by quenched fluorescent substrate assay) were reduced (P<0.05 and P<0.001 respectively). There was a significant positive correlation between BDI-II and Trait Anxiety scores, r=0.0001. In people with the metabolic syndrome, higher BDI-II scores were associated with higher MSNA, 43.2±2 vs 33.3±3 bursts/min (mean±se; P<0.007). Those subjects with higher depression and anxiety scores also exhibited a worse “metabolic profile”: higher anxiety was associated with elevated triglycerides, 1.85 (1.50–2.05) mmol/L vs 1.45 (1.10–1.70) mmol/L (Median [25–75 percentile]; P=0.018), and higher BDI-II scores were associated with increased cholesterol levels, 6.2±0.2 mmol/L vs 5.6±0.2 mmol/L (mean±se; P<0.05). Furthermore, higher BDI-II scores tended to be associated with insulin area under the curve, 11484 (9445–12969) units vs 9084 (7087–10562) units [Median [25–75 percentile]; P=0.058]. Age, gender and blood pressure were comparable in each group. Our data indicate that people with the metabolic syndrome with higher depression or trait anxiety scores exhibit significantly elevated SNS activity and a worse metabolic profile. The central nervous system pathways responsible for the sympathoexcitation in these subjects remain to be determined.

**CARDIAC TISSUE ENGINEERING USING ADIPOSE-DERIVED STEM CELLS**

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Development of regenerative therapies for the heart is hampered by a lack of sources for new cardiomyocytes. Recently treatments that may induce differentiation of cardiac muscle from adult stem cell populations have been identified. In this study, we evaluated co-culture methods for human adipose-derived stem cells (hASCs) with neonatal rat cardiomyocytes (CMs) in vitro and in rat tissue engineering chambers (TEC). ASCs in co-culture exhibited a time-dependent increase in cardiac actin mRNA expression (3.8±0.3x fold) between days 3 and 7. With no further increase at day 14 (3±4x). Immunostaining revealed co-expression of cardiac transcription factors, GATA4 and Nkx2.5, and contractile protein α-smooth muscle actin (a-actinin) and cardiac troponin I (cTnI) in CMs. Real-time PCR demonstrated that STNx rats had increased medullary mas receptor mRNA (P<0.001). ACEII reduced SBP (P<0.01), and inhibited cortical ACE (P<0.001). ACEII was associated with increased cortical and medullary ACE2 activity (P<0.01), and decreased medullary receptor mRNA (P<0.05). These data suggest that dysregulation of ACE2 may contribute to the renal pathology evident following STNx, and suggest that strategies that increase ACE2 and/or its product Ang (1–7) may be useful in the treatment of renal disease.

**305**

**COMPREHENSIVE ANALYSIS OF THE ROLE OF ATP-SENSITIVE POTASSIUM CHANNEL GENES, ABCG9 AND KCNJ8 IN DETERMINING POSTURAL CHANGES IN BLOOD PRESSURE**

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In the Victorian Family Heart Study (VFHS) we have reported that the change in systolic blood pressure from the lying to standing position was 25% heritable and a genome-wide linkage scan suggested evidence of linkage with a region on chromosome 12. In the linked region are two adjacent genes that encode a pore-forming inward rectifier subunit (Kir6.1, encoded by the gene KCNJ8) and an ATP-binding cassette (SUR2, encoded by the gene ABCG9) that are key components of ATP-sensitive potassium channels (KATP). Activation of these channels, which are prominent in vascular smooth muscle cells, produces vasodilation. Experimental disruption of these genes causes vasopositive abnormalities akin to Prinzmetal angina. These channels are also found in brain regions relevant to the baroreflex. Therefore, we hypothesized that...
sequence variants in or around these genes might be associated with postural blood pressure changes. To test this, we comprehensively examined the region by selecting 47 tag single nucleotide polymorphisms (tagSNPs) that represent 242 SNPs spanning the entire genomic region containing KCNJ8 and ABCG9. Using a selective sampling approach, tagSNPs were genotyped in unrelated individuals in whom SBP rose most on standing (n = 150), mean SBP 11.6 mmHg or fell most on standing (n = 150), mean SBP –12.6 mmHg (4 SD). Phenotypic groups were matched for age and sex. Allele and genotype frequencies of each SNP were compared between the two phenotypic groups by chi-square analysis, but we could not identify statistically significant differences for any of the SNPs analysed. Our results strongly suggest that sequence variants in or around these genes are not associated with postural blood pressure changes, and that another gene on chromosome 12 may be responsible for the linkage signal we observed in the VFHS for this blood pressure phenotype.

SODIUM RETENTION DURING EXOGENOUS RENAL NERVE STIMULATION IS ENHANCED IN FAT-FED RABBITS

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The pathway from obesity to hypertension is initiated in part through central nervous system mechanisms. Sympathetic drive is increased, at least partly because of activation of hypothalamic centres by leptin. In turn, the pro-hypertensive effects of increased sympathetic drive in obesity appear to be mediated in part by the renal nerves, since renal denervation blunts the development of obesity-induced hypertension. But the integrated response of the kidney to the sympathetic nerve traffic is still poorly understood. The aim of this study was to investigate whether renal electrical stimulation of the renal nerves (RNS) and/or renal denervation of the renal nerves (RD) could modulate renal function in obesity. We therefore compared the effects of renal denervation and electrical stimulation of the renal nerves on renal function in lean and fat-fed rabbits.

Recent evidence from the Framingham offspring study found that, in subjects of average age 55 years, serum aldosterone levels down through the normal range positively correlated with age and early life. Further, a large number of patients with essential hypertension have autonomous aldosterone secretion, which is known to be associated with increased morbidity and mortality. The role of aldosterone in cardiovascular disease is well established, since recent evidence suggests that aldosterone plays an important role in cell proliferation and heart failure. Since aldosterone is known to have a profound impact on tubular sodium reabsorption. Regardless, our observations raise the possibility that sodium retention in obesity might be driven not only by increased RNSA, but also by increased responsiveness of the kidney to RNSA. These two factors might act synergistically to promote the development of hypertension.

EFFECT OF HYDROGEN SULPHIDE TREATMENT ON PROGRESSION OF ATHEROSCLEROSIS IN APOE-/- MICE FED A HIGH FAT DIET

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Hydrogen sulphide (H2S) is an endogenously produced, gaseous mediator. It reportedly has numerous beneficial cardiovascular effects including anti-inflammatory, anti-oxidant and vasorelaxant properties. Given this, the hypothesis of this study was that H2S would inhibit progression of atherosclerotic disease. Male ApoE-/- mice were fed a high fat diet (21% fat and 0.15% cholesterol) for 16 weeks. For the final 4 weeks they were either left untreated or treated with NaHS. Our results showed that treatment with NaHS was also observed. These results suggest a protective effect of H2S treatment on the vasculature, however further investigation is required.

HARMONIC COMPLEXITY WITHIN THE RENIN ANGIOTENSIN SYSTEM: ROLE OF THE ANG-(1–7)/ACE2/MAS RECEPTOR AXIS

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Characterization of the physiological actions of angiotensin-(1–7) [Ang-(1–7)] provided a new understanding of the role of the RAS in cardiovascular disease by demonstrating that this peptide functioned as an endothelial inhibitor of the pressor and antiproliferative actions of Ang II. Additional insight was gained through the cloning of angiotensin converting enzyme 2 (ACE2) and the identification of the orphan mas receptor as the protein to which Ang-(1–7) binds. ACE2 catalyses the cleavage of Ang II into the vasodilator, natriuretic and anti-proliferative peptide Ang-(1–7). Its role as a cardiac-renal protective hormone was further illuminated by the demonstration that blockade of AT1-R caused a 3-fold upregulation of cardiac ACE2 mRNA parallel with increased plasma Ang-(1–7) and reversal of heart failure. The antiproliferative effects of Ang-(1–7) are mediated by the mas receptor since incorporation of proteins in cardiac myocytes in culture were prevented after administration of an antisense oligonucleotide directed against the mas receptor. The ACE2/Ang-(1–7)/mas receptor axis appears to be critical in the expression of hypertension since in mice. P220 renal hypertension rats both cardiac ACE2 gene expression and cardiac ACE2 activity are significantly reduced both at baseline and after administration of either lisinopril or losartan. Expanding upon these seminal studies, we now provide evidence for the existence of tissues of an alternate precursor for the formation of Ang II and Ang-(1–7). Pro-bradykinin-12 [angiotensin-(1–12, Ang-(1–12)] is cleaved from angiotensinogen by as yet an unidentified enzymes. Ang-(1–12) is found in high concentrations in the heart and the kidney. In addition, cardiac Ang-(1–12) levels are markedly elevated in SHR. Identification of Ang-(1–12) and characterization of the functional role played by the Ang-(1–7)/ACE2/mas receptor axis documents critical differences in the way that tissues process the proteins that lead to the formation of angiotensins. These data underscore the concept that complexity within the biochemical cascade leading to the generation of the biological angiotensins illuminates the harmonious role by which the renin angiotensin system influences homeostasis.

MATERNAL VITAMIN D INSUFFICIENCY IN RATS LEADS TO AN ENLARGED LEFT VENTRICLE WITH MORE CARDIOMYOCYTES IN THE HEARTS OF OFFSPRING

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In recent years there has been a resurgence of vitamin D insufficiency in the community, particularly in women of child-bearing age. Vitamin D insufficiency during pregnancy may affect the development of the heart, since it is known to play an important role in cell proliferation and differentiation. Proliferation of cardiomyocytes occurs mainly prior to birth with postnatal growth of the heart predominantly due to cardiomyocyte hypertrophy. There is clinical evidence to suggest that vitamin D insufficiency leads to cardiomyopathy. The aim of this study was to determine in rat offspring the effect of exposure to vitamin D insufficiency from conception until 4 weeks of age on the development of the left ventricle. Four week old Sprague-Dawley female rats were fed either a vitamin D deficient diet for 6 weeks prior to pregnancy, during pregnancy and throughout lactation. Cardiomyocyte number was determined in the fixed left ventricle of offspring (N=9/group) at 4 weeks of age, using an optical dissector/fractometer stereological technique. In other litters, cardiomyocytes were enzymatically isolated from freshly excised left ventricles to determine the proportion of mononucleated and bionucleated cardiomyocytes (M–B) using a flow cytometer. Cardiomyocyte number in the left ventricle of the vitamin D insufficient offspring was significantly increased (P < 0.01) compared with the control group in males (6.308 ± 0.496 × 10^7 cardiomyocytes and 5.568 ± 0.256 × 10^7 cardiomyocytes, respectively) and females (6.465 ± 0.846 × 10^7 cardiomyocytes and 5.075 ± 0.481 × 10^7 cardiomyocytes, respectively). The number of M–B cardiomyocytes accompanied a significant delay in the maturation of the cardiomyocytes in the hearts of the vitamin D insufficient offspring (P < 0.04). There was also a significant increase (P < 0.05) in cardiomyocyte cross sectional area in the vitamin D insufficient group compared with control offspring. Our findings suggest that exposure to vitamin D insufficiency in utero and early life leads to delayed maturation and subsequent enhanced growth (proliferation and hypertrophy) of cardiomyocytes in the left ventricle. This may lead to altered cardiac function later in life.

RECENT FRAMINGHAM DATA SUGGEST THAT PATIENTS WITH AUTONOMOUS ALDOSTERONE SECRETION WILL BE WELL REPRESENTED IN THE POPULATION OF “ESSENTIAL” HYPERTENSIVES AGED OVER 50 YEARS, AND THAT THEY SHARE A GENETIC BASIS

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Recent evidence from the Framingham offspring study found that, in subjects of average age 55 years, serum aldosterone levels down through the normal range positively correlated with
both blood pressure rise and development of hypertension three and a half years later. This association was stronger in those with urinary sodium/creatinine ratios at or above the median, and non-significant for those below the median. Importantly, renin levels were negatively related to blood pressure rise, suggesting autonomous production of aldosterone, as in primary aldosteronism. All this was consistent with unsuppressible (autonomous) aldosterone secretion in the presence of continued liberal salt intake despite declining nephron population being a risk factor for hypertension development in older subjects. A most interesting further finding was heritability of aldosterone-renin ratio (also showing a strong positive relationship with rising blood pressure) with initial calculations suggesting linkage to chromosome 7p22–22, a region where documentation of linkage is currently under way. Quenouille suggested by us for possible involvement in Familial Hyperaldosteronism type I (FH-I), which is at least five times more common than the glucocorticoid-suppressible Familial Hyperaldosteronism type I (FH-I), for which the mutation is already known and affected subjects are identifiable. Both familial forms are clinically distinguishable from apparently sporadic cases of primary aldosteronism. Linkage studies in families with FH-I from Australia, South America and Italy suggest that the mutation is in the chromosome 7p22 region (LOD score 5.22). It is therefore reasonable to speculate that an inherited tendency to develop autonomous (unsuppressible) aldosterone secretion may be a common feature, which, if understood, is likely to lead to hypertension, given prevailing genetic salt intakes and the “normal” fall in nephron number and GFR with age. Identification of the responsible mutation(s) would make possible identification of those at risk and prevention or cure of hypertension currently labelled “essential”.


042

ACUTE PRENATAL ETHANOL EXPOSURE RESULTS IN ELEVATED MEAN ARTERIAL PRESSURE AND ALTERED RENAL FUNCTION IN OFFSPRING AT SIX MONTHS OF AGE

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Ethanol is a known teratogen, however, the long-term effects of prenatal ethanol-exposure on offspring health has received little attention. Our objective was to investigate the effects of acute ethanol-exposure on mean arterial pressure (MAP) and renal function in male and female ethanol-exposed rats at six months of age. Pregnant Sprague-Dawley rats were administered 1g/kg of ethanol (ETOH) or saline (CON) by gavage on embryonic day 13.5 and 14.5 to mimic binge drinking. At six months of age, conscious MAP and heart rate (HR) was analysed via an invasive tail-cuff catheter. Renal function was analysed in anaesthetised rats via 14C-p-aminohippurate excretion and 90sulin clearance measurements. Pups of both sexes exposed to ethanol were born small but at 6 months of age and body weight (kw) were similar to controls. MAP was significantly higher in both the ethanol-exposed males and females in comparison to their controls (Male CON = 109 ± 1 mmHg, Male ETOH = 116 ± 2 mmHg, P < 0.02). Female CON = 108 ± 2 mmHg, Female ETOH = 119 ± 2 mmHg, P < 0.002 but HR was not different. Glomerular filtration rate was higher in the ethanol-exposed males but lower in ethanol-exposed females in comparison to controls (Male CON = 46.4 ± 0.06 ml/min/gkw, Male ETOH = 46.0 ± 0.16 ml/min/gkw, Male CON = 0.80 ± 0.09 ml/min/gkw, Male ETOH = 0.80 ± 0.08 ml/min/gkw, Female CON = 46.5 ± 0.22 ml/min/gkw, Female ETOH = 46.4 ± 0.20 ml/min/gkw, Female CON = 46.7 ± 0.67 ml/min/gkw, Female ETOH = 46.7 ± 0.67 ml/min/gkw, P = 0.02). Renal blood flow was significantly higher in the ethanol-exposed males and significantly lower in ethanol-exposed females in comparison to controls (Male CON = 4.83 ± 0.63 ml/min/gkw, Male ETOH = 4.65 ± 1.27 ml/min/gkw, Male CON = 0.01, Female CON = 6.22 ± 0.63 ml/min/gkw, Female ETOH 4.70 ± 0.67 ml/min/gkw, P = 0.04). Renal sodium clearance was significantly higher in the ethanol-exposed males, but ethanol-exposed females were similar to controls (Male CON = 17.3 ± 0.19 mmol/min/gkw, Male ETOH = 19.80 ± 0.43 mmol/min/gkw/g, P = 0.05. Female CON = 4.35 ± 0.46 mmol/min/gkw, Female ETOH = 4.59 ± 0.59 mmol/min/gkw in ethanol, P = 0.01). Thus, acute prenatal ethanol exposure leads to an elevation in MAP and sex-specific alterations in renal function. This study is of clinical importance for women who binge drink in pregnancy.

043

LOSARTAN PREVENTS AND REVERSES GLUCOCORTICOID-INDUCED HYPERTENSION

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Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase stimulated superoxide production is increased in adrenocorticotropic hormone (ACTH)-induced hypertension and angiotensin II upregulates NADPH oxidase activity. We previously found that the angiotensin converting enzyme (ACE) inhibitor, Losartan, prevented the development of ACTH-induced hypertension. In this study, we investigated the effect of losartan, an angiotensin II A1 receptor blocker, in glucocorticoid-induced hypertension in rats. Male Sprague-Dawley rats were treated with saline, ACTH (0.2 mg/kg/day s.c.) or dexamethasone (Dex) (10 μg/rat/day) for 13 days. Losartan (in drinking water) or tap water was administered for 17 starting days four days before ACTH or Dex treatment in the prevention study or co-administered for 5 days starting at day 8 of ACTH or Dex treatment in the reversal study. Systolic blood pressure (SBP) was measured by the tail cuff method. Thymus weight was measured as a marker of glucocorticoid action. Aortic ring superoxide production was detected by lucigenin-enhanced chemiluminescence and plasma F2-isoprostane level was measured as a marker of systemic oxidative stress. Results were expressed as mean ± SEM. Neither saline nor losartan/saline treatment changed SBP. SBP was increased by ACTH (from 116 ± 6 to 145 ± 3 mmHg, n = 10, P < 0.001) and Dex (from 114 ± 2 to 145 ± 2 mmHg, n = 10, P < 0.005) and Dex-induced hypertension (122 ± 3 mmHg, n = 10, P < 0.005). Losartan also reversed ACTH- (124 ± 4 mmHg, n = 10, P < 0.005) and Dex-induced hypertension (126 ± 3 mmHg, n = 10, P < 0.005). Both ACTH and Dex decreased thymus weight. Losartan did not affect this marker of glucocorticoid activity. Both ACTH and losartan increased water intake, while Dex decreased it. Neither ACTH nor Dex changed lucigenin-enhanced chemiluminescence significantly and losartan similarly had no effect. In conclusion, losartan prevented and reversed ACTH- and Dex-induced hypertension indicating angiotensin II plays a significant role in glucocorticoid-induced hypertension, possibly through angiotensin II upregulation of NADPH oxidase.

044

RELATIONSHIP BETWEEN AMBULATORY AND CLINIC BLOOD PRESSURE: A HIGH BLOOD PRESSURE RESEARCH COUNCIL OF AUSTRALIA INITIATIVE

GA Head, AS Mihailidou, K Duggan, on behalf of the HBPRCA ABPM Initiative Working Group (NSW, Vic, SA, Qld, Wa)

While management of hypertension is currently by clinical BP assessment, there is increasing need to evaluate 24 hour ambulatory blood pressure (ABP) monitoring. Although ABP equivalents exist for the diagnosis of hypertension, there are no ABP equivalents for target blood pressure (BP) in the management of hypertension. This collaborative initiative was designed to derive a robust algorithm, independent of sex, age and ethnicity, in order to predict reference ABP equivalents for hypertension diagnosis and management targets. Data was collated from 8 centres across five Australian States (n = 2001). All centres used validated devices for 24 hour ABP assessment and seated clinic systolic BP (SBP) and diastolic BP (DBP) was measured by trained non-medical health professionals using a mercury sphygmomanometer, to minimise white coat effect. Standard regression analysis was used to relate clinic and ABP values, with the resultant regression equations being used to generate ABP equivalents for the outcome of grade 1 hypertension (equivalent to 140/90 mm Hg) and target BP (130/85 mm Hg). There were 49.7% male / 50.3% female subjects with average age 55.9 yrs, BMI 29.4 kg/m2 and clinic SBP/DBP of 143 / 84 mm Hg. At each clinic BP target, predicted levels of ABP were quite similar. However, for SBP, the related ABP values were generally higher compared to clinic value (see Table) This contrasts with the PAMELA study which showed much lower ABP values compared to clinic when clinic readings were measured by doctors. Based on these results we would recommend that there is a re-evaluation of the reference ABP equivalents used for management of hypertension.

045

CARDIOVASCULAR RISK FACTOR MANAGEMENT IN AUSTRALIAN GENERAL PRACTICE

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Background: General practitioners (GPs) play a pivotal role in the primary and secondary prevention of cardiovascular (CV) disease, and this can be maximised when management is targeted towards those at highest absolute risk of experiencing a CV event. We aimed to determine the proportion of people in primary care whose CV risk is being treated according to current Australian guidelines. Methods: The Australian Hypertension and Absolute Risk tStudy (AustHEART) was a nationally representative, cluster-stratified, cross-sectional survey among 322 general practitioners (GPs). Each GP was asked to collect data on CV risk factors and their management in 15 to 20 consecutive patients (age ≥55 years) who presented between May and June, 2008. GPs and patients were asked to estimate 5 year CV risk, which was categorised as low (<10%), medium (10–15%), high risk (≥15%) or established CVD and then compared to a central calculated estimate based on submitted data and Framingham risk equation incorporating
The neural mechanisms underlying hypertension are unresolved. It is suggested that elevated sympathetic tone is one contributing factor and this may correlate with an increase in the resting activity of presynaptic neurons, such as the rostral ventrolateral medulla (RVM). We tested the hypothesis that increased sympathetic tone in hypertension may be related to a difference in the activation of g-proteins, specifically Go/o proteins, within the RVM. To test this, we compared the autonomic responses (MAP, HR and temperature) evoked by 8-OHDPAT (5HT-1A agonist, 0.1 and 1mg/kg iv) or oXTM (muscarnic agonist, 0.2mg/kg iv) in conscious telemetry monitored SHR (n=9) and WKY (n=10). The cardiovascular responses to OXTM and the BP response to 8-OHDPAT are sympathetically mediated and evoked predominantly in the RVM. SHR were more sensitive to both 8-OHDPAT and oXTM. SHR exhibited a dose related increase in the duration and degree of depression (p<0.01) and bradycardia (p<0.01) response to 8-OHDPAT compared with WKY. The tachycardic (p<0.001) response to oXTM was also enhanced in SHR. These data suggest that g-protein coupled receptors or downstream proteins are altered in SHR and may be related to the increased sympathetic tone and consequently hypertension. In light of this, we tested the effect of inhibiting Go/o proteins, using pertussis toxin, in the RVM on resting BP. In SHR (n=3), microinjection of pertussis toxin into the RVM had no effect on resting level of BP at any time point following pertussis toxin. The cardiovascular responses to 8-OHDPAT (1mg/kg) were normal at day 2 following pertussis toxin but diminished over the following 4 day period with no cardiovascular response to oXTM (0.2mg/kg) or 8-OHDPAT present by day 6. Therefore, activation of g-protein coupled receptors linked to Gi proteins evokes enhanced responses in SHR versus WKY. In the RVM the effects of ligand binding to GPCR were abolished by Gi blockade in SHR with no effect on resting levels of BP.

**EXPRESSION OF RENALASE, A NOVEL SOLUBLE MONOAMINO OXIDASE IN HUMAN TISSUE**

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In neurons and peripheral tissues, the levels of catecholamines are regulated by three membrane-bound proteins: monoamine oxidase A (MAO-A; MAO-B) and vascular protein-1 (NAP-1). Recently, the discovery of a novel monoamine oxidase, renaissance, was reported. Renalase was demonstrated to be a catecholamine-degrading protein, secreted by the kidney into the blood stream. Interestingly, levels of circulating renalase were shown to be significantly depleted in individuals with end-stage renal disease. It has been postulated that the lack of renaissance in these individuals contributes to elevated levels of circulating catecholamines in these patients. It was also shown that renaissance can function as a negative inotrope, with the ability to significantly alter hemodynamic parameters. We sought further characterise the tissue distribution of renaissance in order to gain a greater understanding of its function in vivo. Analysis of samples obtained from tissue donors revealed that renaissance has a far more extensive representation in human tissue than previously reported and is not restricted to the kidneys and the heart, but is also expressed in peripheral nerves, adrenal glands and in adipose tissue. Furthermore, we have identified several splice variants of the renaissance transcript. These splice variants appear to be tissue-specific and post to a “fine-tuning” of renaissance function. Further investigations into the structural and functional characteristics of renaissance may help to facilitate our ability to diagnose and treat disorders involving an imbalance in the levels of monoamine neurotransmitters.

**TNF-α INFUSION INDUCES A PREECLAMPSIA-LIKE SYNDROME IN PREGNANT PAPAN HAMRADYABOD BABOONS**

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Cytokines and antiangiogenic factors have both been implicated in the pathogenesis of preeclampsia, however the interrelationship of these two systems is yet to be established. The aim of this study was firstly to determine whether a change in circulating cytokines could characterise the tissue distribution of renalase in order to gain a greater understanding of its function in vivo. Analysis of samples obtained from tissue donors revealed that renalase has a far more extensive representation in human tissue than previously reported and is not restricted to the kidneys and the heart, but is also expressed in peripheral nerves, adrenal glands and in adipose tissue. Furthermore, we have identified several splice variants of the renaissance transcript. These splice variants appear to be tissue-specific and post to a “fine-tuning” of renaissance function. Further investigations into the structural and functional characteristics of renaissance may help to facilitate our ability to diagnose and treat disorders involving an imbalance in the levels of monoamine neurotransmitters.

**LEFT VENTRICULAR FILLING RESPONSE TO EXERCISE IS ASSOCIATED WITH EXERCISE-INDUCED CHANGES IN ARTERIAL COMPLIANCE**

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Exercise intolerance due to dyspnoea is common in patients with diastolic dysfunction, implicating the role of raised left ventricular (LV) filling pressure. Exercise E/E' has been validated as a measure of LV diastolic pressure with exercise. The aim of this study was to identify whether changes of arterial compliance are responsible for increases in end-diastolic pressure with exercise. Ten patients with exercise intolerance and raised LV filling pressure during exercise (IFPE; E/E'>15; aged 63±9 years, 10 female) and 10 age and sex-matched controls with normal filling pressure (E/E' <13; aged 61±7 years) were studied. Aortic pulse wave velocity (PWV) was measured at rest whereas simultaneous measurement of E/E' (by echocardiography) and estimated LV afterload (augmentation index; AIx, and central systolic blood pressure; SBP) were recorded by radial tomometry at rest and post maximal treadmill exercise. Resting PWV was higher in patients with IFPE (10.5±3.1 vs. 8.1±1.7 m/s, P<0.05) but there was no cardiovascualr response to oxtremrnx (0.2mg/kg) or 8-OHDPAT present by day 6. Therefore, activation of g-protein coupled receptors linked to Gi proteins evokes enhanced responses in SHR versus WKY. In the RVM the effects of ligand binding to GPCR were abolished by Gi blockade in SHR with no effect on resting levels of BP.

**BARRIERS TO DIAGNOSING AND MANAGING HYPERTENSION**

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Both the initiation of anti-hypertensive medication and the intensification of treatment to therapeutic goals in those with hypertension have been identified as evidence-practice gaps. Few studies have explored the basis for physician behaviour, predominantly relying on...
administered or medical record data. A qualitative method was employed to identify the barriers general practitioners face in diagnosing and managing hypertension, in an Australian setting. Thirty general practitioners (6 focus groups) from the Southern region of Tasmania participated in this study. The focus groups were recorded, transcribed and common emerging themes were analysed by a cumulative process. Once the decision to commence treatment had been made, practitioners found it easy to initiate. However, making a diagnosis and the treatment of patients to target blood pressure levels were viewed as being more difficult. The following barriers were identified: distrust and lack of confidence in the validity of clinical measurements of blood pressure and a distrust of the technology used to measure it; distrust toward the evidence underlying the management of hypertension; adopting a whole person approach to patient care; a perceived increased rate of adverse events amongst the elderly population; lack of internal motivation toward making a diagnosis and reaching target; and perceived patient reluctance to take responsibility for their own health and reluctance to commence and adhere to long-term medications. Lack of skills and lack of knowledge (automated machines for home monitoring and ambulatory blood pressure monitoring), lack of time in consultations, lack of access to timely specialist and allied health input and a government and funding system which supports pharmacological over non-pharmacological approaches were also identified. These findings add weight to the recent debate in the literature that the concept of clinical inertia does not fully capture the complexities of a primary care encounter. The findings indicate that the development of interventions for change for chronic disease management and the writing of guidelines need to take these perspectives into account.

CARDIAC INSULIN-LIKE GROWTH FACTOR-1 (IGF-1) RECEPTOR OVEREXPRESSION PREVENTS DIABETES-INDUCED CARDIAC FIBROSIS AND PRESERVES DIASTOLIC FUNCTION

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IGF-1 promotes physiological cardiac growth and enhances contractile function, and has thus been explored as a target for intervention in an array of animal models of cardiac dysfunction, including diabetes. Diabetic patients exhibit diastolic dysfunction and myocardial remodeling. In the present study, we tested the hypothesis that cardiac-specific overexpression of IGF-1 receptors protects the diabetic heart from remodelling and dysfunction. At 7 weeks of age, type 1 diabetes was induced in male IGF-1 receptor transgenic mice (Tg, n = 9) and non-transgenic mice (Ntg, n = 9) using streptozotocin (STZ, 55 mg/kg, i.p./day for 5 days). Diabetes progressed for 8 weeks, and results were compared to citrate buffer vehicle treated Tg (n = 12) and Ntg (n = 9) sham mice, using two-way ANOVA. Plasma glucose (mM) and glycated haemoglobin (GHb%, n = 9) were not affected by genotype. Diastolic function was assessed on Doppler echocardiography early/late diastolic filling (E/A ratio). Diastolic dysfunction, cardiac fibrosis (%/H11005 (n = 12) and Ntg STZ mice were all attenuated in Tg STZ mice (see table). Cardiac-specific overexpression of IGF-1 receptors however tended to increase all markers of cardiac hypertrophy but was unaffected by L-cysteine and significantly attenuated by carboxy-PTIO (200 μM) and the CGRP antagonist, CGRP (8-37) (1 μM, n = 8). In contrast, DEANo elicited similar concentration-dependent inhibition of ET-1-induced NRCM hypertrophy but was unaffected by L-cysteine and significantly attenuated by carboxy-PTIO (n = 3, P < 0.001). IPA/NO and DEANo (both 1 μM, 10 min) stimulated cGMP accumulation in NRCM (n = 3) and both 10- and fold from control with IPA/NO eliciting more robust response (n = 3). Likewise, IPA/NO and DEANo (both 1 μM, 10 min) stimulated cGMP production in a cell free system to levels 3- and 9-fold of those paired control (P < 0.005; n = 2-4, respectively). Importantly, we used an NO- sensing electrode to demonstrate that IPA/NO, unlike DEANo, does not release NO under our cell culture conditions. Finally, both IPA/NO and DEANo blocked ET-1-induced NRCM superoxide generation (both n = 4, P < 0.001), a key trigger of NRCM hypertrophy. In conclusion, these results provide evidence that IPA/NO prevents NRCM hypertrophy via HNO activation of cGMP. The anti-hypertrophic and antioxidant efficacy of IPA/NO was comparable to IPA/NO, there is no role for extracellular oxidation of HNO to NO- or cGMP-mediated signaling in these IPA/NO actions. Thus HNO donors may represent a novel strategy for the treatment of heart failure, both as stand-alone and add-on therapy to standard care.

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

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Angiotensin II is a key regulator of cardiovascular function that affects the structural and functional properties of novel AT1, AT2, AT1 receptor antagonists containing antioxidant pharmacophores of varying size, in vitro. The AT1, receptor antagonist properties of the novel compounds were tested on angiotensin II-induced positive chronotropy in rat isolated atria. The results from the atrial assays indicated that equimolar concentrations of benzenethione or selenium substitutions of eprosartan retained antagonist potency. However, the addition of the smaller pharmacophore, selenium, caused a greater antagonist effect than the addition of the structurally larger, benzothiophene (EC50 = 7.5 ± 0.1, EC80 = 8.1 ± 0.1 respectively; N = 4). In conclusion, the addition of the antioxidant groups of ebselen, benzenethione or selenium to eprosartan caused a loss of antagonist potency when compared to eprosartan itself (N = 4 each; P < 0.05). The antioxidant capacity of the substituted sartans was examined in an AAPH (2,2-azobis (2-amidinopropane) hydrochloride) induced red blood cell lysis assay (CAST/BL6 mouse isolated red blood cells). Inhibition of lysis over time was quantified by calculating the area under the absorbance curve, with protection of lysis, as seen by quercetin (30 μM) inhibited lysis (N = 8). Neither milfasartan nor eprosartan (10 μM) protected against AAPH/NO-induced red cell blood cell lysis. Similarly, neither benzenethione or selenium analogues of milfarsartan or eprosartan, or the ebselen analogue of milfarsartan (10 μM) inhibited lysis (N = 4; P > 0.05 vs. control). These data suggest addition of antioxidant groups to angiotensin II AT1 receptor antagonists can affect the antagonist properties of the drug in vitro and that the novel compounds do not protect against peroxyl-radical induced red blood cell lysis. Studies looking at protection from other free radical insults may prove to be a more suitable measure of the compounds dual drug effect.

ANTIFIBROTIC EFFECT OF CHRONIC AT-R STIMULATION USING GGP 42112, ON L-NMSP INDUCED FIBROSIS IN MICE

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The renin angiotensin system is important in regulating cardiovascular function, and angiotensin type 1 receptor (AT1-R) stimulation is well known to cause cardiac hypertrophy and
Nitroxyl (HNO) serves as an antioxidant in the cerebral vasculature
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Nitroxyl (HNO), the reduced and protonated congener of nitric oxide (NO), is rapidly emerging as a novel entity with distinct pharmacology and therapeutic advantages over its redox sibling NO. Unlike NO, HNO interacts directly with thios, increases myocardial contractility and is resistant to tolerance development. As such HNO donors may be effective in the setting of heart failure where NO donors have minimal impact. Importantly, HNO also has the potential to serve as an antioxidant and we have preliminary evidence that it suppresses cardiomyocyte superoxide production (1). In the present study we explored the antioxidant properties of HNO in the cerebral vasculature. The HNO donor, angelicin’s salt (A, 1 µM) failed to scavenge O2- generated in the cell free xanthine (100 µM)xanthine oxidase (0.05mU/ml) activity assay (control: 447.9±90.8; AS 507.1±113.3 counts, n=4) in contrast to superoxide dismutase (250U/ml, 42.5±8.6 counts, n=4, P<0.05). Angelicin II (1mM)-stimulated (in the presence of NA/PDH: 100 µM O2- production by isolated cerebral (pooled middle cerebral and basal) arteries from C57BL/6J mice was measured using lucigenin (5 µM)-enhanced chemiluminescence. In preliminary experiments, NADPH oxidase-derived O2- production was ~50% lower in cerebral arteries treated with AS (1 µM) versus control (35690±17960; AS: 24667±12650 counts/mg tissue, n=3). The NO donor, DEA/NO (1 µM) appeared to attenuate O2- production (38383±5200 counts/mg tissue, n=3) to a lesser extent than AS. In summary, HNO does not directly scavenge O2- but may suppress the activity of NADPH- oxidase in the cerebral vasculature. These findings highlight the vasoprotective actions of HNO and its potential as a novel treatment for cardiovascular disorders.

THE 4-YEAR PREDICTED RISK OF TOTAL CARDIOVASCULAR DISEASE IN THE ADVANCE POPULATION: PERFORMANCE OF THE FRAMINGHAM RISK EQUATIONS
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The purpose of the study was to assess the performance of two different Framingham cardiovascular risk functions in a contemporary population of individuals with type 2 diabetes. The 4-year risk of total cardiovascular disease (CVD) was estimated using two Framingham risk equations, for 7502 individuals with type 2 diabetes, who were free of any history of CVD at their enrolment into the Action in Diabetes and Vascular disease: preterax and diamicron-MR for International Health, The University of Sydney (ADVANCE) clinical trial. The risk equations were assessed for the overall performance, discrimination using the area under the receiver-operating curves characteristics (AUC), and the calibration by fits of predicted probability using the Hosmer and Lemeshow statistics (HL). Participants characteristics were – mean (SD) age 65.8 (2.9) years, systolic blood pressure 140.5 (17.3) mmHg, total cholesterol 5.3 (1.5) mmol/L, HDL cholesterol 1.3 (0.4) mmol/L, with median (IQR) known duration of diabetes 7 (3–11) years, HbA1c 7.2 (6.5–8.3%) and triglycerides 1.8 (1.2–2.3) mmol/L. 54% were men, 15% were current smokers, 5% had atrial fibrillation, 65% had treated hypertension and 6% had left ventricle hypertrophy at baseline. The predicted risk of CVD was underestimated by 17.9% (95% confidence interval [95%CI]: 14.6–29.5%) and 20.2% (17.6–23.1%) using the two Framingham equations. The AUC (95%CI) was 0.618 (0.592–0.645) and 0.625 (0.599–0.650) respectively. Within fits of predicted probability, the two equations showed a significant lack of fit, with systematic overestimation of the risk of major CHD (all P for the HL statistics <0.001). Recalibration significantly improved the performance of the 2 equations, but there remained a residual significant lack of fit for one of the models. The results were consistent for men and women, and when the analysis was restricted to patients receiving placebo. Uncritical application of the Framingham and UKPDS risk function to a contemporary population with diabetes is likely to overestimate the risk of total CVD, which has important implications for cardiovascular preventative strategies. Enhanced risk prediction tools are needed to reliably tailor such strategies in people with diabetes.
of 11HPSD2 protection produces oxidative stress, vascular damage and cardiac fibrosis. Salt is also a critical component in the fibrotic response; however the mechanisms behind this pathogenesis remain unclear. Our recent studies have demonstrated that expression of two cardiac genes, angiotensin I converting enzyme 2 (ACE2) and xanthine dehydrogenase (XDH) are selectively up-regulated in mineralocorticoid plus salt-treated animals at the earliest time point in the pathology of MR-mediated cardiovascular disease. The current in vitro study will investigate the regulation of MR transactivation by elevated sodium concentration with the presence of aldosterone and/or cortisone. We determined the effects of a high salt intake on the mechanisms of MR signaling to either aldosterone or cortisol in primary rat cardiac myocytes (HRCC and human embryonic kidney cells (HEK 293)) were transfected with hMR and a MMTV-luc reporter construct. To determine the transactivation of the MR, cells were incubated with each hormone in a dose response manner and elevated sodium concentration (10mM and 20mM) for 24hrs. Preliminary studies demonstrate that elevated sodium content in the culture media modestly enhances aldosterone-mediated MR transactivation. These findings suggest that short-term, elevated sodium may promote MR-mediated inflammatory responses and oxidative damage in cardiovascular pathology. Further studies will investigate the combined effect of salt and oxidative stress on lipid-specific regulation of MR signaling. These will further our understanding of key mediators responsible for the establishment of cardiovascular disease.

EMERGENCE OF AN ENDOTHELIUM-DERIVED HYPERPOLARISING FACTOR (EDHF) COMPONENT IN AORTA FROM THE OBSESE ZUCKER RAT

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The objective of the present study was to compare aortic endothelial function in male obese Zucker rats (OZR, blood glucose 13.7 ± 0.5 mM, n=11, HbA1c 9.6 ± 0.2%, n=6), an established model of type 2 diabetes, with that in age-matched control lean Zucker rats (LZR, blood glucose 10.5 ± 0.5 mM, n=9; HbA1c 6.2 ± 0.1%, n=5). Endothelium-intact thoracic aortic rings from male 25-week Zucker rats were suspended in organ baths for isometric force recordings in the presence of 10 μM indomethacin. In aortic rings precontracted with phenylephrine (0.1–3 μM), endothelium-dependent relaxations to acetylcholine (Ach) and endothelium-independent relaxations to sodium nitroprusside were not significantly different (P<0.05, ANOVA, n=6–9) between LZR and OZR. Addition of 100 μM nitro-L-arginine methyl ester (L-NAME) and 1 μM ODQ to block the nitric oxide (NO) pathway abolished Ach responses in LZR (Emax, reduced from 87 ± 4% preconstriction to 3 ± 5%, n=6). In contrast, l-NAME and ODQ only partially blocked Ach responses in aortic rings from OZR (Emax reduced from 75±2% to 23±4%, n=9); the residual response was blocked by completely- and intermediate-conductance Ca2+–acti- vated K+ channels with 1 μM TRAM-34 (E30 reduced from 6 to 3%). In a separate series of experiments, relaxations to Ach in aortic rings from LZR (n=3) were resistant to inhibition by 1 μM amapin and 1 μM TRAM-34 but were abolished after further incubation with L-NAME and ODQ. On the other hand, Ach responses in OZR were reduced (Emax, decreased from 76±1% to 60±6%, n=3) after blockade of the EDHF pathway with amapin and TRAM-34, and the residual response was abolished by l-NAME and ODQ. These results suggest that although the overall endothelium-dependent relaxation to Ach is unchanged in the aorta from type 2 diabetic OZR, there is an alteration in the balance of NO and EDHF components of the response; endothelial NO is reduced in the OZR but the reduction appears to be compensated by the emergence of an EDHF component which is not normally present in this vascular tissue.

GLUCOCORTICOID ACTIVATION OF CARDIAC MINERALOCORTICOID RECEPTORS IN EXPERIMENTAL MYOCARDIAL INFARCTION

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Clinical trials have shown a pathophysiological role for mineralocorticoid receptor (MR) activation in essential hypertenison and cardiac failure; aldosterone is commonly assumed to be responsible for non-physiological MR activation, plasma levels of aldosterone were in the low normal range. The aim of the current study was to determine whether cardiac MR are activated by glucocorticoids during tissue damage in the perfused rat heart. Sprague Dawley rats were anesthetized, hearts isolated and subjected to regional ischemia (30 min.) followed by reperfusion (2.5 hr). Aldosterone and Cortisol with or without spironolactone (SP), Tempol and GR/PR antagonist RU486 were added to the perfusate. To determine the excitatory role of endogenous glucocorticoids, rats were transfected with miR-133 were differentially expressed. We have identified novel mRNA and miRNA interactions and genes that directly correlate with cardiac function and may represent novel targets for the treatment of heart failure. 1. McMullen JR et al. 2003 PNAS:14:12355–60; 2. Shiioi et al. 2000. Embo J 19:2537–48.

EXPONENTIAL DAMAGE LEADS TO INCREASED WHOLE BODY INSULIN SENSITIVITY IN ADULT MALE AND FEMALE RAT OFFSPRING

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Many studies link poor fetal growth with the ‘programming’ of metabolic syndrome later in life. We investigated the effect of intrauterine growth restriction (IUGR), due to maternal protein restriction, on whole body insulin sensitivity in adult rat offspring. Female WKY rats were fed either a normal protein diet (NPD, 20% casein) or low protein diet (LPD, 8.7% casein) during pregnancy and lactation. After weaning, all offspring were maintained on normal rat chow. Tail-cuff systolic blood pressure was measured from 24 to 32 weeks of age. Whole body insulin sensitivity was determined using a euglycemic-hyperinsulinaemic clamp at 32 weeks of age in both male (n=7–9) and female (n=7–9) offspring. Plasma and pancreatic insulin levels were also measured. Body weights in the LPD offspring were significantly reduced compared to NPD offspring; no difference were observed in blood pressure. Interestingly, the whole body insulin sensitivity index (SI) was significantly increased (P<0.0001) in the IUGR group (0.52±0.19 and 6.69±0.20, males and females respectively) compared to controls (2.75±0.18 and 4.03±0.30, males and females respectively). This was accompanied by a significant reduction in plasma insulin in male and female LPD offspring (P=0.0231) but no difference in pancreatic insulin. Overall females were more sensitive to insulin (P=0.0046) compared to males. In conclusion, IUGR, due to maternal protein restriction, unexpectedly appears to lead to improved glucose tolerance in adult rats at 32 weeks of age. Whether this programmed improved glucose metabolism persists into old age is yet to be determined.

MICRONORNA AND MRNA EXPRESSION PROFILING IN A MOUSE MODEL OF MYOCARDIAL INFARCTION WITH ENHANCED OR DEPRESSED PI3K ACTIVITY

RCY Lin1, RBH Williams1, XJ Du2, XM Guo2, H Kiriazis3, MJ Cowley4, HJ Speirs1, JWG Dawes1, JR McMullen3, Ramaciotti Centre for Gene Function Analysis, University of New South Wales, 4Molecular Systems Biology Group, John Curtin School of Medical Research, Australian National University, 5Baker IDI Heart & Diabetes Institute, Melbourne, Victoria, 6Peter Wills Bioinformatics Centre, Garvan Institute of Medical Research

Cardiac hypertrophy is an increasing epidemic in Western society. The aberrant growth of the heart can lead to heart failure, and can be induced either by physiological stimuli eg postnatal development or chronic exercise; or by pathological stimuli eg pressure/volume overload or in response to myocardial infarction (non-infarcted region). The pathological condition is associated with fibrosis, cardiac dysfunction and increased morbidity and mortality. Previous work (McMullen et al) showed distinct signalling cascades induce pathological and physiological cardiac hypertrophy (1). One of the mechanistic processes involving the heart to enlarge in response to physiological stimuli while maintaining normal or enhanced function is the p110alpha isoform of phosphoinositide 3-kinase (PI3K). It plays a critical role in the regulation of developmental heart growth (2) but the underlying regulation is not well understood. Activation of genes involved can be a potential tool to augment physiological growth and improve cardiac function of the failing diseased heart. Thus, we investigated the transcriptome profiles of both mRNA and microRNA (miRNA) in 6 mouse models associated with progression of the disease in the non-infarcted “hypertrophied” left ventricle. Transgenic (Tg) mice expressing a constitutively active (ca) PI3K (p110α) mutant specifically in the heart have a 6.5-fold increase in PI3K activity and a 20% increase in heart weight/body weight (HW/BW) ratio of non-transgens (Ng). Dominant negative (dn) PI3K (p110α) mutant had a reduced PI3K activity (77%) and HW/BW ratio (20%). The role of PI3K in a different setting of heart failure was examined by subjecting adult Ng, dnPI3K and CaPI3K Tg mice to myocardial infarction (MI) for 8 wks. Global mRNA and miRNA expression were identified in Ntg-sham, dnPI3K-sham, CaPI3K-sham, Ntg-Mi, dnPI3K-Mi and CaPI3K-Mi (n =4 each) using Affymetrix GeneChip® Mouse Genome 430 2.0 arrays and Agilent miRNA arrays. Interestingly, GO terms belonging to mitochondria genes were overrepresented. Cardiac specific mir-1, miR-208 and miR-133 were differentially expressed. We have identified novel mRNA and miRNA interactions and genes that directly correlate with cardiac function and may represent novel targets for the treatment of heart failure. 1. McMullen JR et al. 2003 PNAS:14:12355–60; 2. Shiioi et al. 2000. Embo J 19:2537–48.

063

064

2008 HBPRCA Presentations

1111

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ISOLATED CLINICAL HYPERTENSION IS ASSOCIATED WITH ELEVATED TWO-HOUR GLUCOSE POST ORAL GLUCOSE TOLERANCE TEST

CA Martin1, SSH Chen1, JD Cameron2, BP McGrath1, 1Centre for Vascular Health, Monash University and Dandenong Hospital, 2La Tracte University, Melbourne

Elevated two-hour glucose post oral glucose tolerance test has been shown to be associated with incident hypertension in the AusDiab study. Isolated clinical hypertension (ICH) is considered to be an intermediate condition between normotension and hypertensive on a progressive observational study we have been examining whether ICH, defined on two separate 24mth ambulatory blood pressure (ABP) recordings, can be better characterised by defining functional and circulatory biomarkers, autonomic function, glucose tolerance and insulin resistance. Smokers and diabetics were excluded in this study. Subjects had baseline measurements of ambulatory blood pressure, central pulse wave velocity (PWVc), glucose tolerance, insulin resistance and autonomic function tests. Results are given as mean±SD. For adjusting for mean arterial pressure (MAP), PWVc was significantly increased in the HT group only. Mean glucose, 2h post 75g oral dextrose, was similarly elevated in ICHT and HT groups and remained significantly different, compared to the NT group, after adjusting for waist measurement (p=0.02). Fasting and AUC insulin were not significantly different across groups after adjusting for waist measurement. Autonomic dysfunction was detected in 3 of the ICHT subjects, in 1 of the HT subjects, and 0 of the NT subjects. At the one year visit five ICHT subjects (26%) were HT on ABP and two others were on anti-HT medication.

Conclusion: Raised 2h post-load glucose, autonomic dysfunction and progression to HT all indicate that ICHT is not a benign condition.

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1Centre for Vascular Health, Monash University and Dandenong Hospital, 2La Tracte University, Melbourne

NEUROPROTECTIVE EFFECT OF AN AT1 RECEPTOR AGONIST AFTER STROKE INDUCTION IN SPONTANEOUSLY HYPERTENSIVE RATS

CA McCarthy1, A Vinh1, JE Callaway2, RE Widdop1, 1Dept of Pharmacology, Monash University, Melbourne, 2Dept of Pharmacology, University of Melbourne

Aims: On the basis of A\textsubscript{T}\textsubscript{1} receptor knockout studies, the A\textsubscript{T}\textsubscript{1} receptor has been implicated to be neuroprotective although this premise has not been directly tested. Therefore, we have examined the potential neuroprotective role of the A\textsubscript{T}\textsubscript{1} receptor following intracerebroventricular (icv) administration of A\textsubscript{T}\textsubscript{1} receptor agonist CGP42112 after stroke induction in a conscious rat model of stroke.

Methods: Spontaneously hypertensive rats (SHR) were treated with CGP42112 at 4 time points up to 72 hours post-stroke (cumulative dose 3.5µg) either alone or in combination with the AT\textsubscript{2} receptor antagonist PD123319 (cumulative dose 125µg). A focal reperfusion model of stroke was induced in conscious rats by administering endothelin-1 adjacent to the middle cerebral artery through a surgically implanted cannula. Behavioural tests were used to assess the severity of neurological deficit as a result of the ischemic event. Cortical and striatal infarct volumes were measured 72 hours post stroke.

Results: Blood pressure was unaffected by treatments. CGP42112 reduced infarct volume post stroke (P<0.05). This effect was negated with the co-administration of PD123319. These results were consistent with the behavioural findings, indicating that CGP42112 reduced motor deficit on the lateral beam test at 24 and 72 hours post stroke (P<0.05).

Conclusions: These results confirm our previous data demonstrating that pre-treatment with an A\textsubscript{T}\textsubscript{1} receptor agonist is neuroprotective independent of any changes in blood pressure. Thus, the current study has shown for the first time that central A\textsubscript{T}\textsubscript{1} receptor stimulation following a cerebral accident is neuroprotective in a conscious rat model of stroke.

DIETARY FRUCTOSE-INDUCED CARDIAC HYPERTROPHY AND OXIDATIVE STRESS IS ASSOCIATED WITH SUPPRESSED MYOCARDIAL SIGNALLING THROUGH AKT

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Dietary fructose intake has increased considerably in recent decades, in parallel with an increase in the incidence of insulin resistance. The impact of fructose on the heart is poorly understood. The aim of this study was to determine the cardiac effects of a 12 week 60% high fructose dietary intervention in C57Bl/6 male mice. Blood pressure was measured by tail cuff and systemic insulin sensitivity was estimated by glucose tolerance test. Hearts were collected for measurement of ventricular weight index (VWI) and myocardial production of superoxide (lucigenin chemiluminescence). Phosphorylation states of signalling proteins in myocardial tissue were analysed by western blot. VWI was increased by 22% in the fructose fed mice vs. control, 12.1±6.5 mmol/L vs. 0.6±2.2 mmol/L, respectively. Likewise, the induction of a high arterial state by a standardized alerting stimulus (placing mice in novel home cages) caused similar pressor responses in both groups on either diet. Administration of the A\textsubscript{1} antagonists prazosin markedly decreased MAP in both groups. HSD did not affect this response in AT\textsubscript{1}−/− mice (35.5±4 mmHg), but abolished it in AT\textsubscript{1}−/− animals (1.6±0.6 mmHg). These results indicate that AT1 receptor deficiency increases ΔMAP when combined with high fructose diet but is not associated with any changes in arterial blood pressure or arterial baroreflex function. The present study demonstrates that a 12 week dietary fructose intervention induces cardiac hypertrophy associated with oxidative stress. Fructose-induced insulin resistance is apparent both
systemically and intrinsic to the myocardium. These findings suggest that cardiac insulin resistance may play a role in fructose-induced cardiac pathologies in the absence of haemodynamic disturbance.

**ULTRADIAN RHYTHMS IN ARTERIAL PRESSURE AND HEART RATE ARE PART OF THE BASIC REST-ACTIVITY CYCLE IN RATS**

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The homeostatic set-point paradigm of arterial blood pressure regulation emphasizes baroreceptors in keeping arterial blood pressure constant. In the last 20 years there have been reports that AP and heart rate oscillate in an ultradian manner, with peak values occurring every 1–2 hours throughout both phases of the circadian cycle. We have re-investigated this question in Sprague-Dawley rats instrumented under isoflurane or ketamine/xylazine anaesthesia with intra-arterial catheters that also recorded behavioral activity, and (different rats) with temperature probes in brown adipose tissue (BAT temperature), extra-durally within the cranial cavity (brain temperature) and in the peritoneal cavity (body temperature), EEG electrodes for recording hippocampal theta (5–8 Hz) rhythm. Recordings were carried out in undisturbed conscious unrestrained animals, at ambient temperatures of 22–26°C, with food and water available ad libitum, and with the 24-hour day divided into 12 hours of dark and light. Arterial pressure and heart rate increased by 18±5 mmHg every 98±16 for 30±5 min (mean±SD) minutes during the dark (active) phase of the circadian cycle. At the same time, with a cross correlation –0.9 and no phase difference, heart rate increased by 5±3 beats/min. These increases were highly correlated with corresponding rhythms in BAT temperature, brain temperature, body temperature and behavioral activity (rearing, sniffing, moving about the cage) and with 5–8 Hz theta power in the hippocampal EEG. The EEG rhythms were approximately 5 minutes ahead of all the other rhythms. One interpretation of the findings is that rats actually heat up their brains during periods of activity, and that increases in arterial pressure and heart rate function to distribute the BAT heat to the brain and to skeletal muscles. After the discovery of rapid eye movement (REM) sleep, Kleitman proposed that as a Basic Rest-Activity Cycle (BRAC) persists through the wakeful phase of the 24-hour circadian cycle. Our results support this view, and suggest that ultradian rhythms in arterial pressure and heart rate are part of the BRAC, and substantially independent of baroreceptor-related homeostatic regulation of arterial pressure.

**INCREASED BLOOD PRESSURE LOAD DURING BEREAVEMENT: POTENTIAL MECHANISM FOR INCREASED CARDIOVASCULAR RISK**

AS Mihailidou1,2, T Buckley1,3, M Spinaze1, D Roche1, M Bramwell1, S McKinley1,2, MC Morel-Kopp2, C Ward1,2, R Bartrop3, G Toffler1,2, 1Royal North Shore Hospital, 2University of Sydney and 3University of Technology, Sydney

Bereavement is associated with increased risk of cardiovascular disease (CVD), particularly in the acute period, although the physiological changes for this increased risk have not been defined. Ambulatory blood pressure monitoring has not been previously reported in bereavement. The results support this view, and suggest that ultradian rhythms in arterial pressure and heart rate are part of the BRAC, and substantially independent of baroreceptor-related homeostatic regulation of arterial pressure.

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**HIGH DENSITY LIPOPROTEINS INTERACT WITH MONOCYTES AND ENDOTHELIAL CELLS TO DIFFERENTIALLY REDUCE CELL ADHESION**

AJ Murphy1, D Sviridov, K Woodall, J Chin-Dusting, Baker IDI Heart and Diabetes Institute, Melbourne

High Density Lipoprotein (HDL) has many cardio-protective effects, most notably its role in reverse cholesterol transport. The anti-inflammatory function of HDL, particularly its ability to inhibit cell-cell adhesion has recently commanded a high level of interest. In this study, we investigated the relative contribution of HDL induced monocyte vs endothelial cell anti-adhesive properties. To examine the effect of HDL on monocyte driven cell adhesion over time, human monocytes were isolated from whole blood and stimulated with PMA for 15 mins before addition of HDL (50 µg/mL) over an increasing amount of time (0–8hrs) and then allowed to adhere to a TNF-α activated monolayer of human coronary arterial endothelial cells (HCAECs). To examine the effect of HDL on endothelial cell driven adhesion, HCAECs were pre-stimulated with TNF-α for 4 hrs and then incubated with HDL (50 µg/mL) over time (0–8hrs) and PMA activated monocytes were allowed to adhere for 15 mins. To examine the potential for an additive effect of HDL on both monocytes and endothelial cells, cells were stimulated as above and incubated with HDL for various amount of time before adhesion was assessed. To examine the effect of HDL on monocyte driven cell adhesion over time, human monocytes were isolated from whole blood and stimulated with PMA for 15 mins before addition of HDL (50 µg/mL) over an increasing amount of time (0–8hrs) and then allowed to adhere to a TNF-α activated monolayer of human coronary arterial endothelial cells (HCAECs). To examine the effect of HDL on endothelial cell driven adhesion, HCAECs were pre-stimulated with TNF-α for 4 hrs and then incubated with HDL (50 µg/mL) over time (0–8hrs) and PMA activated monocytes were allowed to adhere for 15 mins. To examine the potential for an additive effect of HDL on both monocytes and endothelial cells, cells were stimulated as above and incubated with HDL for various amount of time before adhesion was assessed. To examine the effect of HDL on monocyte driven cell adhesion over time, human monocytes were isolated from whole blood and stimulated with PMA for 15 mins before addition of HDL (50 µg/mL) over an increasing amount of time (0–8hrs) and then allowed to adhere to a TNF-α activated monolayer of human coronary arterial endothelial cells (HCAECs). To examine the effect of HDL on endothelial cell driven adhesion, HCAECs were pre-stimulated with TNF-α for 4 hrs and then incubated with HDL (50 µg/mL) over time (0–8hrs) and PMA activated monocytes were allowed to adhere for 15 mins. To examine the potential for an additive effect of HDL on both monocytes and endothelial cells, cells were stimulated as above and incubated with HDL for various amount of time before adhesion was assessed. To examine the effect of HDL on monocyte driven cell adhesion over time, human monocytes were isolated from whole blood and stimulated with PMA for 15 mins before addition of HDL (50 µg/mL) over an increasing amount of time (0–8hrs) and then allowed to adhere to a TNF-α activated monolayer of human coronary arterial endothelial cells (HCAECs). To examine the effect of HDL on endothelial cell driven adhesion, HCAECs were pre-stimulated with TNF-α for 4 hrs and then incubated with HDL (50 µg/mL) over time (0–8hrs) and PMA activated monocytes were allowed to adhere for 15 mins. To examine the potential for an additive effect of HDL on both monocytes and endothelial cells, cells were stimulated as above and incubated with HDL for various amount of time before adhesion was assessed.
MECHANISMS FOR CHANGE IN AORTIC AUGMENTATION WITH AGE

M. Narasimhay7,1, A. Adjil2,3, M. O’Rourke1,2,4, Department of Cardiology and Victor Chang Cardiac Research Institute, St. Vincent’s Clinic, Sydney; 2Faculty of Medicine, The University of New South Wales, Sydney; 3Australian School of Advanced Medicine, Macquarie University, Sydney

Augmentation index (AIa) is a widely used measure of wave reflection and aortic stiffness. It rises with age in a curvilinear manner whereas central augmentation pressure (AP) and pulse pressure (PP) from which it is determined (as AIa=AP−PP) rise linearly with age. Apparent flattening of AIa over age 60 has been attributed to decreased peripheral wave reflection in older subjects. We sought a purely mathematical explanation of this phenomenon—a that two positively sloped linear equations with different intercepts on the y-axis yield a curvilinear change when one is divided by the other. Data were from 1601 patients attending a previously described outpatients clinic and aged from 25 to 93 years. The rise in AP and PP could be described by straight lines (Figure), whereas their ratio as AIa was curvilinear and approximated the change described outpatients clinic and aged from 25 to 93 years. The rise in AP and PP could be described by straight lines (Figure), whereas their ratio as AIa was curvilinear and approximated the change when age is as described in other studies (Figure). Change in AIa with age cannot simply be attributed to decrease in peripheral wave reflection. The phenomenon described here must be considered together with change in shape of the left ventricular ejection pattern with age.

MEASURING AUSTRALIAN SALT INTAKES

J. Webster1, E. Dunford1, J. Keogh1, T. Beard1, J. Chalmers1, S. Corbett2, GA Nosworthy1, B. Neal1,1 The George Institute for International Health, University of Sydney, 2CSIRO Human Nutrition, 3The Menzies Research Institute, 4Sydney West Area Health Service, University of Sydney, 5Deakin University

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 6 grams per day. Whilst generally accepted to be too high, there is substantial uncertainty about current levels of salt consumption in Australia. The objective of this study was to summarise the available data estimating salt intake in Australia. A systematic review of the literature was undertaken to identify all published studies that have reported an estimate of salt consumption for adults in Australia. Estimates based on dietary recall methods and urinary sodium excretion were included where n≥5 and measurements were not confined to specific ethnic groups. Twelve studies conducted between 1997 and 2008 were identified: 10 utilised 24-hour urine collection and 2 used dietary recall methods. Only one study sought to estimate the salt consumption of the general population and that used dietary recall. The remainder were studies of sub-samples of the population recruited for other reasons. Estimated mean salt intake from the different studies ranged between 3.2 and 7.9 g/day (Sodium (Na) 55–135 mmol/day) for dietary recall, and between 6.5 and 12 g/day (Na 111–205 mmol/day) with 24-hour urine collections. Estimated salt consumption was generally higher in men than women. The available data are insufficient to reliably estimate the level of salt consumption in the Australian population. Bias associated upon the assay techniques used on the non-representative sample of most of the studies make it impossible to reliably estimate mean salt consumption in Australia. The planned National Diet and Physical Activity Survey offers a unique opportunity to determine salt consumption in Australia using 24-hour urines in a sub-sample of those surveyed.

TIME TO STOP TREATING HYPERTENSION

B. Neal. Senior Director, George Institute for International Health, Professor of Medicine, University of Sydney, Chair, Australian Division of World Action on Salt and Health

Hypertension is one of the disease risks most well known to patients and doctors and one of the chief conditions for which consultations are held and medications prescribed. It is perhaps therefore surprising that blood pressure remains the second leading cause of disease burden in Australia. In part because of the inadequate treatment of hypertension. But more fundamentally, it represents the disconnect between what we know about how blood pressure causes disease, and the approach we take to its management. The continuous nature of the association between blood pressure and risk has been established for decades. Despite this, the treatment of hypertension, which views blood pressure as an exposure that causes disease only above a particular threshold (usually 140/90 mmHg), remains the predominant approach to preventing blood pressure-related disease. Unfortunately, half of all death and disability caused by blood pressure occurs in people who don’t have hypertension. Hypertension programs mostly fail to address the risks in these non-hypertensive people. Furthermore, many people with hypertension go unrecognized and large numbers of those that do receive a diagnosis are untreated or fail to reach targets. As a consequence, hypertension control programs have rather limited potential for the prevention of the disease burden caused by blood pressure. If Australia is to do better it must fundamentally change its approach. A strategy based primarily upon hypertension control exposed in a hypertension guideline is no longer an acceptable option. It is widely understood that programs that assign blood pressure lowering treatment to patients on the basis of risk, or that seek to shift population blood pressure levels offer enormously greater potential. Moreover, both approaches are highly plausible and might avert a greater proportion of the blood pressure-related disease burden at lower cost. Australian scientists have played a key role in the scientific discoveries underpinning this evidence. It is now time to translate this knowledge into practice and accrue the health benefits for Australians.
Albinumuria and reduced kidney function are risk factors for macrovascular disease in type 2 diabetes (T2DM) however the extent to which these risk factors are independent of each other is uncertain. The Action in Diabetes and Vascular disease: preterAx and diaminor-MR Controlled Evaluation (ADVANCE) study examined the effects of routine blood pressure lowering on clinical outcomes in T2DM. We assessed the relationship between baseline levels of urinary albumin-to-creatinine ratio (UACR) and glomerular filtration rate (GFR), and the risk for macrovascular outcomes in 10,640 patients for whom UACR and serum creatinine measurements were available at baseline. Baseline UACR levels were in the microalbuminuric (30–300 μg/mg) and macroalbuminuric (≥300 μg/mg) range in 27% and 4% of patients, respectively. The baseline prevalence of GFR ≤60 ml/min/1.73 m² was 19%, 62% of whom were normoalbuminuric. There was a log-linear increase in the risk of macrovascular events with greater baseline UACR and lower baseline GFR levels (both p trend <0.001). The multivariate-adjusted risks of macrovascular events increased by 31% (95% confidence interval 23–40%) for every doubling of baseline UACR and by 120% (45–233%) for every halving of baseline GFR after adjustment for regression dilution bias. There was no evidence of any interaction between the effects of higher UACR and lower GFR (p interaction >0.3).After adjustment for potential confounding factors, the presence of both macroalbuminuria and reduced GFR at baseline was associated with a 2.1-fold higher risk of macrovascular events, when compared to individuals with normoalbuminuria and GFR ≥60 ml/min/1.73 m².

Heart Research Institute

Albinumuria and reduced kidney function are risk factors for macrovascular disease in type 2 diabetes (T2DM) however the extent to which these risk factors are independent of each other is uncertain. The Action in Diabetes and Vascular disease: preterAx and diaminor-MR Controlled Evaluation (ADVANCE) study examined the effects of routine blood pressure lowering on clinical outcomes in T2DM. We assessed the relationship between baseline levels of urinary albumin-to-creatinine ratio (UACR) and glomerular filtration rate (GFR), and the risk for macrovascular outcomes in 10,640 patients for whom UACR and serum creatinine measurements were available at baseline. Baseline UACR levels were in the microalbuminuric (30–300 μg/mg) and macroalbuminuric (≥300 μg/mg) range in 27% and 4% of patients, respectively. The baseline prevalence of GFR ≤60 ml/min/1.73 m² was 19%, 62% of whom were normoalbuminuric. There was a log-linear increase in the risk of macrovascular events with greater baseline UACR and lower baseline GFR levels (both p trend <0.001). The multivariate-adjusted risks of macrovascular events increased by 31% (95% confidence interval 23–40%) for every doubling of baseline UACR and by 120% (45–233%) for every halving of baseline GFR after adjustment for regression dilution bias. There was no evidence of any interaction between the effects of higher UACR and lower GFR (p interaction >0.3).After adjustment for potential confounding factors, the presence of both macroalbuminuria and reduced GFR at baseline was associated with a 2.1-fold higher risk of macrovascular events, when compared to individuals with normoalbuminuria and GFR ≥60 ml/min/1.73 m².

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of the Australian patient population drawn from de-identified Pharmaceutical Benefits payment records from January 2003 to December 2006. Only Concessional patients were included because many AHT products fall under the General copayment. Initiation means a dihydropyridine (DHP) script after no DHP script for 6 months. Almost 6,000 of these initiated patients, where the DHP was added to another AHT, were selected and their persistence assessed. Persistence on a DHP was highest when the DHP was added to an ACE followed by an A2RA while persistence on a DHP was poorest when the DHP was added to a diuretic. Median persistence (months [95% CI]) when adding a DHP differed by AHT type: ACE 22 months [18–25], A2RA 19 months [16–22], Beta Blocker 14 months [11–16], Alpha blocker 10 months [4–28] and Diuretic 9 months [7–10]. In terms of optimal treatment persistence, lercanidipine seems to be the best DHP to add to either an ACE or an A2RA. Median persistence was highest when a lercanidipine was added to an ACE (27 months [18–33]) followed by an A2RA (19 months [12–28]). Lercanidipine "add on" persistence seems to be superior (Figure 1) compared with the addition of amlodipine to either an ACE (15 months [12–18]) or an A2RA (12 months [10–20]). Prescribers need to assess which CCB molecule best supports NHF treatment goals.

**Figure 1.** DHP persistence when added to an ACE or an A2RA.

**Inferred Mortality Differences Between Dihydropyridine Antihypertensives**

M Ortiz1, G Calcino2, Solvay Pharmaceuticals, Sydney, HI Connections, Canberra

PBS claims data provided by Medicare Australia can be used to identify poor compliance (persistence) as well as infer mortality. Treatment persistence describes how long patients remain on a therapy (until stopping). There are significant differences in persistence between dihydropyridine (DHP) antihypertensive (AHT) drugs. Death can be inferred from the PBS claims database using the last date of any PBS script, provided that date is at least 6 months before the date of data extraction. This analysis is based on all scripts supplied to a one in ten sample of the Australian patient population drawn from de-identified PBS Claims between January 2003 and December 2006. Only Concessional patients were included because most AHT products fall under the General copayment. For persistence curves, initiation means a new DHP script with no script for a DHP in the previous 6 months. For mortality curves, death was inferred if no PBS script was obtained by a patient between 1 January 2007 and 30 June 2007. Date of inferred death was defined as the date of the last PBS script. Three different groups of patients initiated on a DHP have been considered: "AHT naïve"; "Switch" and "Add on" patients. There seems to be an association between cessation of AHT treatment and inferred mortality for patients newly initiated on a DHP. The patients initiated on the other DHPs had an 88% increase in inferred mortality (HR 1.88 [1.77 to 2.00]) compared with patients initiated on lercanidipine (Log-Rank p<0.00001). The patients initiated on the other DHPs (Figure 1) also had a 75% greater inferred mortality (HR 1.75 [1.60 to 1.91]) compared with patients initiated on lercanidipine (Log-Rank p<0.00001). Inferred mortality of patients initiated on lercanidipine was consistently lower compared with other DHPs for all three initiation groups ("AHT naïve", "Switch" and "Add on").

**Figure 1.** Inferred Mortality by DHP molecule.

**Time to Re-start After Stopping Antihypertensive Treatment**

G Calcino1, M Ortiz2, HI Connections, Canberra, Solvay Pharmaceuticals, Sydney

PBS Claims data for antihypertensive treatments (AHTs) provided by Medicare Australia have been used to identify patients who restart treatment after ceasing AHT treatment. This analysis is based on all scripts supplied to a one in ten sample of the Australian patient population drawn from de-identified Pharmaceutical Benefits payment records from January 2003 to December 2006. Only Concessional patients were included because most AHT products fall under the General copayment. Time to restart to any AHT or proportion restarting each AHT treatment, were at 50% greater risk of CHD (risk ratio 1.47, 1.30 – 1.66) and in those with macroalbuminuria (risk ratio 2.17, CI 1.87 – 2.52). Sensitivity analysis indicated no uncertainty. A meta-analysis of cohort studies was conducted. MEDLINE and EMBASE were searched for studies reporting an age or multivariate adjusted estimate and standard error of the association between proteinuria and either stroke or coronary heart disease (CHD). Studies were included if the majority of the study population had known glomerular disease or were the recipients of renal transplants. Two independent researchers extracted the estimates of association between proteinuria, microalbuminuria, or macroalbuminuria and the risk of each endpoint. These were combined using a random-effects model. 26 cohort studies involving 199,949 individuals and 7,117 coronary events (27% fatal) reported on the relationship between proteinuria and CHD, and 10 studies involving 140,231 participants and 3,266 strokes described the relationship with stroke. The presence of proteinuria was associated with an approximate 50% increase in coronary risk (risk ratio [RR] 1.47, 95% confidence interval [CI]: 1.33 – 1.61) and a 70% increase in the risk of stroke (RR 1.79, CI 1.39 – 2.10), after adjustment for known risk factors. There was evidence of a dose response relationship between increasing albuminuria and CHD: individuals with microalbuminuria were at 50% greater risk of CHD (risk ratio 1.47, 1.30 – 1.66) and in those with macroalbuminuria the risk was more than doubled (risk ratio 2.17, 1.87 - 2.52). Sensitivity analysis indicated no important differences in pre-specified subgroups. These data confirm a strong and continuous association between proteinuria and subsequent risk of cardiovascular disease, and suggest that proteinuria should be incorporated into the assessment of an individual’s cardiovascular risk.

**Cardiac Anti-fibrotic Effects of Angiotensin (1–7) Mimetin in Aged Mice**

DS Phay, ES Jones, RE Widdop, Department of Pharmacology, Monash University, Melbourne

We have previously shown that combined AT1R inhibition and Ang (1–7) treatment caused an anti-fibrotic effect in hearts of aged SHR. Moreover this effect was reversed in the presence of...
the AT<sub>2</sub> receptor antagonist, PD123319, suggesting that Ang (1–7) mimetic, AVE 0991, has been developed and shown to mimic the effects of Ang (1–7) on the endothelium (Wiener et al., 2002), to improve endothelial function and to prevent the development of severe hypertension and end-organ damage in SHRs (Benter et al., 2006). However, to date, there have been no studies looking at the chronic effects of AVE 0991 in an aged setting. Thus, the aim of this project was to determine the effects of chronic AVE 0991 treatment in aged mice.

Aged C57Bl/6J mice (20 months) were treated for eight weeks with either Angiotensin (1–7) (24 μg/kg/hr, s.c.) or the angiotensin (1–7) mimetic AVE0991 (24 and 120 μg/kg/hr, s.c.). Low dose AVE 0991 was also given with either the AT2R receptor antagonist A779 (48 μg/kg/hr) or the AT<sub>R</sub> antagonist, PD123319 (10 mg/kg/d).

At the conclusion of treatment, hearts and aortas were removed to examine cardiovascular structure. Indices of cardiac left ventricular to body weight ratio, cardiomyocyte cross sectional areas of aorta (medial wall-cerebral) and aortic wall thickness were not influenced by any drug treatments. However, AVE 0991, Ang (1–7) and Candesartan alone all significantly decreased cardiac fibrosis (0.005% Picrorosuv Red). Interestingly, the anti-fibrotic effect of AVE 0991 was reversed by simultaneous treatment with either AT79, or PD 123319, suggesting that Ang (1–7) may be working via the MAS and/or the AT<sub>R</sub> receptor in the heart. Therefore, angiotensin (1–7) and AVE0991 reversed the marked cardiac fibrosis that was already established due to aging in mice, but without any effect on cardiac and vascular hypertrophy. These anti-fibrotic effects may occur due to stimulation of both angiotensin (1–7) and/or Mas receptor- and AT<sub>R</sub> receptor- mechanisms.

MATERNAL OBERITY IN RABBITS ELEVATES OFFSPRING BLOOD PRESSURE AND EVOKES SELECTIVE LEPTIN RESISTANCE

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Leptin is secreted from white adipose tissue in proportion to its mass and acts in the hypothalamus to decrease food intake and increase sympathetic nerve activity (SNA) and blood pressure. A characteristic of adult-onset obesity is selective leptin resistance, where obese individuals respond to leptin treatment with a blunted or absent appetite suppressant effect of leptin but not to the pressor effects. Maternal obesity is implicated as a risk factor for the development of obesity and hypertension in offspring. The aim of this study was to determine if offspring from obese mothers become selectively leptin resistant. 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 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

CHANGES IN ENDOTHELIN-DERIVED HYPERPOLARISING FACTOR (EDHF) IN RENAL ARTERIES FROM TYPE-2 DIABETIC RATS

M Bansal, JJ Reid,° School of Medical Sciences, RMIT Univ, Melbourne

This study compared endothelial function in 3rd-order renal arteries from male type-2 diabetic obese Zucker rats (OZR, blood glucose: 13.4 ± 0.6 mM, n = 22; HbA<sub>1c</sub> 6.1 ± 0.1%, n = 11) and control lean Zucker rats (LZR, blood glucose: 9.8 ± 0.5 mM, n = 21; HbA<sub>1c</sub> 6.1 ± 0.1%, n = 25) at 26 weeks of age. Endothelial function was assessed in a Mulvaney-Halpern wire myograph in the presence of 10 μM indomethacin. Relaxations to endothelium-dependent (endothelin-1 and the independent dilator sodium nitroprusside) were not different in arteries from diabetic and control rats. Blockade of the nitric oxide (NO) pathway using nitro-L-arginine methyl ester (100 μM) and ODQ (1 μM) significantly reduced (P < 0.05, ANOVA) relaxations to ACh in OZR (Emax reduced from 84 ± 1% to 64 ± 1%, n = 26; P < 0.05, compared to LZR). ACh-induced relaxations to ACh in OZR (Emax reduced from 86 ± 1% to 62 ± 1%, n = 26; P < 0.05). The residual relaxation was abolished in both groups of rats (n = 5–6) by inhibition of large-, intermediate- and small-conductance Ca<sup>2+</sup>-activated K<sup>+</sup> channels using charybdotoxin (0.1 μM) and apamin (1 μM). The NAME/OO- resistant response was not altered by the NO synthase inhibitors L-NMMA (10 μM) and L-NAME (1 mM), suggesting that the NAME/ODQ resistant response associated with endothelial NO is reduced in the OZR but there appears to be a compensatory increase of the EDHF component which may be related to an upregulation of connexin 43-associated gap junction activity.

TWO-YEAR CLINICAL OUTCOMES FOR HYPERTENSIVE PATIENTS AT HIGH-RISK OF ATEROTHROMBOSIS IN AUSTRALIAN GENERAL PRACTICE – LOCAL RESULTS FROM THE REACH REGISTRY

CM Reid,° 2 Adam1, M Nelson°, 3 G Conroy°, 2 D Chey1,° L Shiel1, A Soman,° F de Looze° on behalf of the REACH Registry Investigators,° 1CCSE Therapeutics, Monash University, Melbourne;° 2Department of General Practice, University of Tasmania, Hobart;° 3Liverpool Hospital, Sydney;° 4Dept of Cardiology, Finders University,° 5Sand-sant-avents, Sydney;° 6Department of General Practice, University of Queensland

Background: Atherothrombosis is the leading cause of cardiovascular morbidity and mortality in Australia. However, little is known in Australia about the current cardiovascular event rates in stable patients managed in general practice. The aim of the current study is to report on 2-year cardiovascular event rates in patients with established cardiovascular disease or with multiple risk factors.

Methods: As part of the international REACH Registry, subjects at high risk of atherothrombosis based on a) the presence of multiple risk factors, b) overt coronary artery (CAD), c) cerebrovascular or peripheral vascular disease or d) are undergoing anticoagulant therapy were reviewed. Two-year cardiovascular event review. Two-year rates of CV death, myocardial infarction, stroke as well as hospitalisation for cardiovascular procedures are reported. All rates are adjusted for age and gender using Cox proportional hazards models.

Results: 2,873 were recruited from 281 general practitioners around Australia of which 2734 (95%) were included in the 2 year follow-up. The all-cause mortality and CV death rate was 4.6% and 2.5% respectively. The combined CVD death, non-fatal MI, stroke and hospitalisation rate was 16.52%. Even for those with multiple risk factors only, the two-year combined event rate was 7.52%. The highest combined event rate was in those with PAD (28.9%) and those with disease identified in three locations (CAD, CVD, and PAD) 57.6%.

Conclusion: Community based patients with stable atherothrombotic disease experience relatively high two year event rates. Where disease is evident in more than one location, the 2 year risk of CV events is increased.

THE AT,R-MEDIATED DEPRESSOR RESPONSE TO ANG II IN FEMALES IS ESTROGEN DEPENDENT

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It is now clear there is a vasodilatory arm of the renin angiotensin system that directly opposes the classical vasoconstrictive actions of Angiotensin II (Ang II). We have previously shown that in males, a low dose of Ang II, which has no effect on mean arterial pressure (MAP) in males, female MAP decreased by ~10 mmHg. In addition, we have also shown that this depressor response seen in females is mediated by the Ang II type 2 receptor (AT,R). Furthermore, estrogen has been shown to directly interact with the renin angiotensin system shifting the balance of vasodilator to vasoconstrictor components towards vasodilation. Therefore we hypothesised that the depressor response to low dose Ang II in females is estrogen dependent and hence we aimed to investigate the role of estrogen in this Ang II induced depressor response in females. Females underwent either sham or ovariectomy surgery and were treated with either a placebo or estrogen pellet. MAP was measured using radiotelemetry in response to a two week infusion of saline or Ang II (50 ng/kg/min s.c.). As observed previously, MAP significantly decreased in females treated with Ang II (10–2 mMg), a response that was abolished by ovariectomy (increased 4±2 mmHg) and restored with estrogen replacement (~65–20 mmHg). In control rats, the AT,R expression was greater in ovariectomised and ovariectomised-replaced replaced Ang II treated females (~4 fold) compared to saline treated animals with a concomitant increase in AT,R (4 fold) in Ang II treated ovariectomised rats. That is, in females, estrogen treatment decreased the AT,R:AT, R ratio. We suggest that in females, estrogen dependent mechanisms counteract the pressor actions of Ang II by enhancing the recently discovered vasodilator pathways of the renin angiotensin system. This highlights the potential for these vasodilator pathways as therapeutic targets, particularly in women.

ALTERED RENALASE SECRETION IN NEUROGENIC HUMAN HYPERTENSION

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Renalase, a novel soluble mammalian oxiase, is secreted by the kidneys and involved in regulation of catecholamine metabolism. In patients with end stage renal disease, plasma levels of renalase have been reported to be substantially reduced and possibly to be linked with high circulating noradrenaline levels characteristic of the condition. We aimed to assess whether renalase is implicated in neurogenic forms of human hypertension. Radiotracer dilution methodology and simultaneous arterial and renal vein sampling was applied to measure renal noradrenaline (NA) spillover and renine-kinin system. The renal vein was performed using a polyclonal anti-renalase antibody. We studied 5 patients with neurogenic hypertension and one normotensive healthy control subject. Renal NA spillover was substantially higher in the 5 hypertensive patients compared to the control subject (55 ± 53 vs 66 ng/min). Western blot analysis showed that renalase expression in the renal vein was significantly reduced in patients and controls. In the healthy control subject an arterio-venous step up of renalase across the kidney was evident, indicative of release of renalase from the kidney. In contrast, such a step up was not apparent in the arterial and renal vein samples from 4 of 5 patients with neurogenic hypertension. Preliminary findings from this small patient series indicate that the plasma concentrations of renalase may be decreased in a subset of patients with neurogenic hypertension, thereby potentially contributing to their increased noradrenaline spillover and elevated blood pressure level.
UNCOUPLING OF SYMPATHETIC NERVE ACTIVITY AND ENDOTHELIUM-DEPENDENT VASODILATION IN EARLY ESSENTIAL HYPERTENSION

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Regulation of vascular tone is the result of a complex interplay between a variety of systems including the sympathetic nervous system and endothelium derived factors. We aimed to assess whether alterations in the interaction between sympathetic nerve traffic and endothelium-dependent vasodilation may be related to increased vascular tone. Resting multi-unit sympathetic nerve firing rates were measured at the peroneal nerve by microenereuography. Endothelium dependent vasodilation was assessed by measuring forearm blood flow (FBF) using venous occlusion plethysmography at rest and during infusion of acetylcholine at a dose of 37 μg/min. Our study cohort consisted of 13 untreated young hypertensive patients (EH), 12 young healthy volunteers with a strong family of essential hypertension (PFH), and 14 young healthy volunteers without a family history (NFH). Resting muscle sympathetic nerve activity (MSNA) was higher in EH compared to PFH and NFH (46 ± 18 vs. 31 ± 13 bursts/min, p = 0.05). Resting FBF was similar in all three groups. Forearm blood flow after intra-arterial infusion of acetylcholine was lower in EH and in PFH compared to NFH (10.7 ± 2.4 vs. 11.1 ± 4.0 vs. 14.8 ± 3.6 ml/min/100ml; p < 0.05). In NFH, MSNA correlated significantly with the forearm blood flow response to acetylcholine (r = 0.53; p < 0.01), i.e., the higher the MSNA the higher the response to acetylcholine. In contrast, no evidence for such a relationship was evident neither in PFH (r = 0.24; p = 0.44) nor in EH (r = −0.17; p = 0.58) in young healthy control subjects, the vasconstrictor effects of muscle sympathetic nerve traffic appear to be counteracted by an adequate response to endothelium derived nerve stimuli to maintain normal vascular tone. This coupling between sympathetic activity and endothelium derived factors appears to be altered in EH and PFH, thereby potentially contributing to blood pressure changes characteristic of early essential hypertension.

5-HD INHIBITS LEVOSIMENDAN IN HUMAN ISOLATED ATRIA POST-HYPOXIA

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Levosimendan is postulated to have an important cardiac action by protecting myocyte function during ischaemia. This study investigated the effects of levosimendan on contractile function in human isolated atrial tissue, and to test whether mitochondrial KATP (mKATP) activation is involved in this response following hypoxia and reoxygenation. Human atrial trabeculae were mounted in an organ bath electrically paced and contractile force measured for baseline stabilisation (BL). The contractile response to isoprenaline was measured after tissues were subjected to normoxia, or hypoxia (H) and reoxygenation (R), and taken to be an indicator of cytoprotection. In addition, atrial tissue was pretreated (P) with levosimendan and the mKATP antagonist 5-hydroxydecanoate (5-HD). In levosimendan pretreated tissue contractile force was increased compared to other tissue on reoxygenation (p < 0.05). The maximal response to isoprenaline was significantly increased with levosimendan compared to the hypoxia control time (0.92 ± 0.09 versus 0.70 ± 0.09 g, P < 0.01). This contractile response was blocked by 5-HD (0.62 ± 0.11 g, P < 0.01). After hypoxia-reoxygenation levosimendan preserves the contractile force to isoprenaline, via mKATP channel activation.

TARGETING THE GENES THAT REGULATE AGING: A CLINICAL UPDATE

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The diet known as calorie restriction (CR) is currently the most robust way to improve health and slow aging in mammals. The fact that CR works on most species, even microorganisms, implies a conserved underlying mechanism. We work under the hypothesis that CR extends lifespan because it is a mild biological stressor that activates conserved longevity genes, one of which is the sirtuin deacetylase SIRT1. The SIRT1 gene is thought to have evolved in primordial eukaryotes to help them survive adverse conditions and to have been conserved to the present day in fungi, plants and animals. Interestingly, SIRT1-activating molecules (STACs) produced by stressed plants, such as resveratrol, can activate SIRT1 in yeast and animals, extending their lifespan by a mechanism analogous to CR. Effects include increased mitochondrial biogenesis, increased endogenous neuroprotection, reduced inflammation and improved cardiovascular health. Arteries of mice treated with resveratrol are considerably protected from arterial stiffness, cell death, and inflammation than untreated animals. The physiology of the 27 month old mice is more akin to mice ~9 months young. Since 2000, synthetic STACs have now been synthesized and potency has been enhanced ~1000 times over resveratrol. The latest data from mouse longevity studies and ongoing human clinical studies with resveratrol and synthetic STACs will be presented.
ALISKIREN DOES NOT CAUSE PARADOXICAL RISES IN BLOOD PRESSURE

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Diuretics, angiotensin converting enzyme inhibitors (ACEs), angiotensin receptor blockers (ARBs), and aldosterone receptor blockers (ARBs) have all caused rises in plasma renin concentration. High levels of renin have been reported with DRs such as aliskiren (ALI). This has prompted speculation that, in some patients, aliskiren’s blockade of plasma renin activity (PRA) could be overwhelmed, and this could lead to a paradoxical rise in BP. In order to examine this hypothesis, we performed a meta-analysis of the frequency of increases in mean sitting systolic and diastolic BP (SBP and DBP) above predefined thresholds in 8 double-blind, randomized trials (treatment duration 4–12 weeks), conducted in 4877 patients with mild-to-moderate hypertension, in which ALI was compared with placebo (PBO), or with an active comparator. The frequency of BP rises was evaluated for the pooled monotherapy groups: (1) PBO; (2) ALI 300 mg; (3) high-dose ARB (losartan 300 mg, valsalol 300 mg and furosemide 100 mg); (4) ACEi, ramipril (RAM) 10 mg and (5) diuretic, hydrochlorothiazide (HCT) 25 mg. A subset of patients with PRA measurements (n = 538) was analyzed to identify whether BP rises on aliskiren were associated with a rise in PRA (LPAH ≥ 0.1 ng/mL/h). Overall, the frequency of BP rises (1%) with ALI 300 mg was low, and similar to or lower than that observed with comparable doses of ARBs, RAM or HCT (Table). No patient who received ALI 300 mg, whose SBP or DBP rise by more than 10 mmHg or 5 mmHg respectively, demonstrated a rise in PRA > 0.1 ng/mL/h. In conclusion, we found no association between increased BP and increased PRA during treatment with aliskiren. As the frequency of BP rises with aliskiren was considerably less than that with placebo, and similar to that observed with treatment with other antihypertensive drugs, we found no evidence that aliskiren causes paradoxical rises in BP.

Bilateral adrenal hyperplasia (BAH), the most common form of primary aldosteronism (PAL), is an important clinical issue. We examined blood pressure (BP) and biochemical responses in patients with BAH undergoing unilateral ADX and sought predictive parameters. From 1984–2004, 684 patients were diagnosed with PAL within our Center. Of the 51 who underwent unilateral ADX, 40 who were clinically followed for ≥12 (median 26) months, lacked other secondary endocrine or renal causes of hypertension, and were not treated with aldosterone antagonists post-operatively at their request, were included in the analysis. Reasons for surgery included intolerance of, or inadequate hypertension control with, aldoster antagonist medications (n = 22), strong patient preference (n = 9) or presence of an adrenal mass lesion. Unilateral ADX was performed in 50% of patients post-glucose and by similar magnitude in both groups (P = 0.05). In conclusion, IR MetS subjects have increased resting MSNA and a blunted sympathetic neural responsiveness to oral glucose compared with IS MetS subjects which is not meditated by differences in skeletal muscle vasodilation or cardiac BR. Since SNS activity contributes to the postprandial rise in energy expenditure, a blunted response in IR subjects could promote weight gain. We have reported the high precision and specificity of our new LC-MS/MS method for plasma aldosterone. Radiomimassays (RMAs) have lower precision and specificity, but have been clinically useful in establishing the high incidence of primary aldosteronism (PALT) and in its management. We used the new LC-MS/MS assay to examine whether our current RIA (DPC Coat-a-Court) might have led to either over- or under-diagnosis of PAL. Fludrocortisone suppression test (FST) involves serum samples at 8000 ng/mL (normal range) and 1000 (24th post basal and after 3 and 4 days of fludrocortisone and oral salt loading). On day 4, FST PA of ≥ 165 pmol/L is diagnostic of PAL. Results by LC-MS/MS for 16 patients with PAL were included in the analysis. In conclusion, RIA and MS/MS day 4 upright PA concentrations were

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103

104
We have previously shown that the angiotensin peptide, Angiotensin (Ang) (1–7), can mediate vaso- and athero-protective effects when given chronically for 4 weeks to apolipoprotein E-deficient (ApoE−/−) mice. These effects appeared to be mediated via activation of either the specific Ang (1–7) receptor, known as the Mac receptor, or the AT1 receptor (AT1R). Therefore, in the current study, we used specific agonists for the two receptors: AVE0991, an Ang (1–7) non-peptide mimetic, and CGP42112, an AT1R agonist and determined their effects on the progression of atherosclerosis. All treatments were investigated using the ApoE−/− mouse model, with assessment of atherosclerotic lesion and endothelial function. Mice were fed a high fat diet for 16 weeks, with chronic treatment administered in the final 4 weeks via osmotic mini-pumps. Treatments were either: saline (vehicle), AVE0991 (24 μg/kg/hr) or CGP42112 (60 μg/kg/hr) alone, as well as in combination with either an AT1R antagonist, PD123319 (416 μg/kg/hr), or the Mas antagonist, A779 (48 μg/kg/hr). Compared with vehicle-treated ApeE−/− mice (max relaxation (Rmax) = 55.08 ± 1.35%), chronic treatment with AVE0991 and CGP42112 significantly improved endothelial function (Rmax = 75.08 ± 1.36% and 77.7 ± 1.62%, both P < 0.001). The effect of AVE0991 was attenuated by either AT1R or MasR blockade, whereas the CGP42112 effect was reversed by the AT1R antagonist only. In vessel segments adjacent to those used for vascular reactivity studies, supernatide levels (assessed by DHE fluorescence) were significantly reduced, and eNOS immunoreactivity was increased, with both agonists when compared to vehicle treated mice (P < 0.01). Both AVE0991 and CGP42112 significantly decreased lesion development (Immunohistochemistry media score (IRM) = 0.43 ± 0.12 and 0.46 ± 0.14, respectively) compared with vehicle controls (IRMs = 1.2 ± 0.18 and 1.25 ± 0.18; both P < 0.05). All of these effects of AVE0991 were reversed by either AT1R or MasR antagonists, whilst AT1R blockade alone inhibited the effects of CGP42112. This study indicates that both AVE0991 and CGP42112 can improve endothelial function as well as reduce atherosclerotic lesion progression via either the AT1R or the Mac receptor, or both. AVE0991 displayed a similar profile to that of Ang (1–7), with apparent activation of both receptors mediating vaso- and athero-protective effects. This study highlights the protective nature of non-AT1 receptors in atherosclerosis.

Effect of Sepiapterin on Glucocorticoid-Induced Hypertension

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Sepiapterin is converted to intracellular tetrahydrobiopterin (BH4) by sepiapterin reductase enzyme and incubation with sepiapterin restored endothelial dysfunction in aorta from dexamethasone-treated rats. However, supplementation of BH4 failed to prevent dexamethasone- or adrenocorticotropic hormone (ACTH)-induced hypertension in rats in vivo. The aim of the present study was to investigate the effect of sepiapterin supplementation on ACTH- and dexamethasone-induced hypertension in rats.

Male Sprague-Dawley rats were treated with sepiapterin (5 mg/kg/day i.p.) or its vehicle (saline) for 5 days. Saline, ACTH (0.2 mg/kg/day s.c.) or dexamethasone (10 μg/kg/day) were co-administered from day 5 for 11 days. Systolic blood pressure (SBP) was measured by the tail-cuff method. Thymus weight was measured as a marker of glucocorticoid activity.

Results are expressed as mean ± SEM. SBP was increased in vehicle + ACTH (from 117.3 ± 3.5 mmHg, n = 12; P < 0.001) and vehicle + dexamethasone treated rats (from 114.4 ± 3.3 mmHg, n = 13; P < 0.0005) but not in vehicle + saline treated rats (from 114.3 ± 3.3 mmHg, n = 12, n.s.). Sepiapterin did not change SBP in ACTH- or dexamethasone-treated rats compared with vehicle treated rats (SBP vehicle + saline 119.3 ± 3.3 mmHg, n = 12; vehicle + ACTH 135.3 ± 3.5 mmHg, n = 12 and vehicle + dexamethasone 133.3 ± 3.3 mmHg, n = 13; P > 0.05). Thymus weight was significantly decreased in both ACTH and dexamethasone compared with saline treated rats. Sepiapterin did not affect this marker of glucocorticoid activity.

In conclusion, sepiapterin does not prevent ACTH- or dexamethasone-induced hypertension. These data do not support a role for eNOS uncoupling in the genesis of glucocorticoid-induced hypertension.

Light Exercise Central Blood Pressure: A Correlate of Left Ventricular Mass That Is Simpler Than 24 Hour Ambulatory Blood Pressure Monitoring

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Twenty four hour ambulatory blood pressure monitoring (24 ABPM) is the gold standard for assessing blood pressure (BP) control because data derived from this technique outweights all other BP measures for predicting target organ damage. Central BP during daily activity may be a stronger determinant of cardiovascular risk, but this cannot be derived from 24 ABPM. This study aimed to compare the correlations of 24 ABPM and light exercise estimated central BP (mmHg) with left ventricular mass (LV mass). Forty healthy subjects aged 57–7 years with no history of renal disease, a negative stress echocardiogram, and not treated for hypertension were studied. All subjects underwent 2D echocardiography for determination of LV mass (indexed; g/m²), resting brachial BP, 24 ABPM, and estimated central BP by radionuclide during graded cycle ergometry. Central systolic BP (SBP) was determined from the radial second systolic peak (P2) as well as from the derived central pressure waveform. Mean LV mass index was 37.1 ± 8.2 g/m² (range 23.1 to 55.1 g/m²) and 24 ABPM SBP 133 ± 11 mmHg (range 114 to 153 mmHg). As expected, mean 24 ABPM SBP was significantly associated with LV mass index (r = 0.35, P = 0.04) but not with SBP during light exercise at 50% heart rate reserve were the strongest correlates of LV mass index (other variables tested included age, gender, body mass index, and all 24 ABPM measures). The strength of correlations with LV mass index were compared between light exercise radial SBP and 24 ABPM SBP by 2 statistic, which was of borderline significance (Z = 1.91; P = 0.065). On multiple linear regression analysis, radial SBP but not 24 ABPM SBP, was independently associated (β = 0.60; P = 0.001) with LV mass index after accounting for all other variables mentioned above (model R² = 0.41; P < 0.001). We conclude that exercise radial SBP is associated with LV mass independent of 24 ABPM SBP. This single measure of exercise radial tonometry (which takes about 15 minutes) may be a superior test to determine BP control.
Hypertension in the spontaneously hypertensive rat (SHR). Expression plasmids with RNA promoter and 3 different RNAi sequences targeting AT1R DNA were constructed. RNAi plasmids were transfected into cultured SHR vascular smooth muscle cells for assessment of AT1R silencing. The RNAi transcript that was specific for AT1R silencing and which resulted in the highest potency was used for in vivo experiments. At a preliminary operation telemetric blood pressure transducers were positioned in the abdominal aorta of 8 month old male SHR for long-term measurement of blood pressure. Arterial blood pressure was recorded weekly and values averaged over 24 hours. After a 2 week blood pressure monitoring period RNAi plasmid (n=6), a control plasmid containing green fluorescent protein (GFP, n=6) or saline (NaCl, n=6) was administered to the SHR by tail vein injection. The animals were followed for a further 8 weeks. Additional animals were treated with RNAi plasmid or saline and euthanased at different time points over 8 weeks for determination of tissue AT1R expression using RT-PCR. In cultured VSMCs the RNAi plasmid with highest potency showed a reduction in AT1R expression of approximately 75% and 60% using fluorescence microscopy or Western blot respectively. In animals injected with RNAi plasmid blood pressure was lowered 1 week after injection with maximal reduction at 4 weeks. Blood pressure returned to resting levels by 8 weeks. There was a correlation between reduction in tissue expression of AT1R particularly in liver and heart fall in blood pressure. Brief small changes in liver function tests were observed on day 1 following injection of plasmid. Significant blood pressure reduction was achieved in 8 month old male SHR for approximately 2 months by RNAi induced AT1R gene silencing. RNA inhibition of AT1R expression can be effectively induced using RNAi technology and offers a potential new approach to blood pressure control approaches.

**SESAME LIGNANS AND THEIR EFFECT ON IN VITRO 20-HYDROXYEICOSATETRAENOIC ACID GENERATION**

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20-Hydroxyeicosatetraenoic acid (20-HETE) is a major product of cytochrome P450 (CYP450) catalysed metabolism of arachidonic acid. 20-HETE has vasoactive and natriuretic properties although its exact role in human hypertension remains unknown. Previous studies suggest CYP4F2 and CYP4A11 are the major human CYP450 isoforms responsible for 20-HETE synthesis in both liver and kidney. Furthermore, recent studies suggest that sesamin and sesamolin, the major lignans derived from sesame seeds, may be potent inhibitors of CYP4F2 activity. In this study we investigated the inhibitory effects of sesame lignans on 20-HETE generation. Data shown are mean±SE of three independent experiments. In human renal microsomes, sesamin and sesamolin dose-dependently inhibited 20-HETE synthesis (IC50 of 5.3±0.5 and 4.2±1.2 μM, respectively). In contrast, other structurally similar phytoestrogens such as pinoresinol and secoisolariciresinol showed weak or no inhibitory activities for 20-HETE synthesis at 50 μM. Enterolactone and enterodiol, which are major in vivo metabolites of sesame lignans also showed markedly reduced inhibitory activities compared to their parent compounds (at 50 μM, 26.6±4.3% and 18.5±1.6% inhibition compared to vehicle treated controls, respectively). Similar results were observed in human liver microsomes. In experiments using recombinant microsomes expressing human CYP450 isoforms, sesamin showed selective inhibition of CYP4F2 (IC50 of 1.9±0.1 μM) compared to CYP4A11 (IC50>150 μM). Our studies suggest that sesame lignans may inhibit 20-HETE production in humans via inhibition of CYP4F2. Future studies need to investigate if supplementation with sesame or its purified lignans lead to altered 20-HETE production in human and possible related biological effects.

**RISK REDUCTION OF LIFESTYLE-RELATED DISEASES IN ADOLESCENTS WITH SOY OR FISH – RICH JAPANESE TRADITIONAL MEALS**

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Since our previous trial, “well-balanced lunch once a day” was proven to reduce cardiovascular risk factors effectively in Japanese businessmen with mildly elevated risks in 4 weeks, we carried out a 5 week nutritional intervention study with soy or fish-rich lunch in female college students. After the questionnaire and a physical checkup, 164 female junior college students aged 18–24 were randomized into three groups: C (ordinary lunch), S (soy lunch), F (fish lunch). Lunches contained the same energy content (630 kcal) with 700 mg of DHA. The intervention lasted 5 weeks, and all lunches contained the same energy content (630–700 Kcal), same amount of protein and fat and 3.5–4.0 g of salt. Health survey including blood sampling and 24-hour urine collection was carried out prior to and after the intervention. Triglycerides/TG of S group was significantly decreased from the baseline (P<0.01) and significant inter-group differences were observed between C and F groups (P<0.05). Since body fat% was significantly increased in C group from the baseline (P<0.01), significant inter-group difference was observed in the amount of change between C and S groups (P<0.01). Even though this intervention was carried out from summer to winter and body fat% normally increased, there was no significant increase in body fat% in S and F groups. It was observed that biomarkers were within normal range because participants were young, but 9.1% of blood samples showed a high values (>221) in total cholesterol. In these groups with high total cholesterol level, it was observed that diastolic blood pressure (DBP) and total cholesterol was significantly decreased from the baseline (P<0.05, P<0.01) in Figure.

In conclusion, the TG as well as body fat% were decreased by taking well-balanced lunch with soy or fish for 5 weeks, although it was within the normal range. Therefore, the diet enriched with soy or fish, common in Japanese traditional foods, can effectively reduce the risk of lifestyle-related diseases, even in female adolescent Japanese, exposed to Westernized dietary custom in recent years.
HYPERTENSION, TRADITIONAL RISK FACTORS AND ORAL HEALTH IN TANZANIAN MAASAI

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Objective: Previously we reported the association of oral health with an increased risk of hypertension in middle-aged Tanzanian women. The purpose of this study is to investigate the association between hypertension and oral health in Tanzanian Maasai. Design and Methods: The present study was conducted in Tanzania according to the WHO-coordinated Cardiovascular Diseases and Alimentary Comparison (CARDIAC) Study protocol. Four hundred thirty-five people (244 men and 229 women) aged 46–58 years, including 143 Maasai (59 men and 84 women), were recruited randomly for blood pressure (BP) and anthropometrical measurements, blood sampling, 24-hour urine collection and the examination of dental status and periodontal status. Results: Systolic BP (SBP) in Maasai and the other subjects were 115 ± 19 mmHg and 130 ± 23 mmHg, respectively. Diastolic BP (DBP) was 75 ± 15 mmHg, respectively. The average of SBP and DBP was significantly lower in Maasai than the other subjects (p < 0.001). Body mass index (BMI) in Maasai averaged 20.8 ± 3.3, much lower than that of the other subjects (24.4 ± 5.8, p < 0.0001). Tooth loss (number of tooth missing) in Maasai and the other subjects was 2.6 ± 1.7 and 2.5 ± 3.3. Lower permanent central incisors of Maasai were removed traditionally in their childhood. Periodontal status in Maasai averaged 2.05 ± 0.55, was much better than that of the other subjects (2.42 ± 0.57, p < 0.0001). In multiple regression analysis, the severity of periodontal disease and tooth loss was significantly correlated with hypertension in women except for Maasai. Conclusion and Discussion: Despite the association of oral health and BP observed in middle-aged Tanzanian women, there was no association between oral health and BP in Maasai whose dental condition was appeared better because of their habitual brushing, traditional social habit of clean teeth and dairy intake of traditional yogurt recently known to have immuno-potentiating effect.

INTERACTIVE BLOOD PRESSURE EFFECT OF SODIUM AND TAURINE IN 7 JAPANESE POPULATIONS FROM CARDIAC STUDY

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Taurine (T) excretion in 24-hour urine (24U), a biomarker of seaweed intake, has been closed up by CARDIAC (Cardiovascular Diseases and Alimentary Comparison) Study covering 61 populations in 25 countries, because 24U T was proven to be inversely related significantly with the age-adjusted mortality rates of coronary heart diseases and stroke. T was only one factor that might contribute to the prevention of both cardiovascular diseases among 5 diet related factors (24U T, 24U sodium (Na), serum total cholesterol, body mass index and 24U magnesium). On the other hand, classical experimental studies on salt-sensitive stroke-prone spontaneously hypertensive rats (SHRSP) and DOCA-salt rats showed dietary T administration attenuated the development of hypertension and stroke. Therefore, we analyzed the association of 24U T and 24U Na in 7 Japanese populations taking both dietary factors at the higher level compared with 54 other CARDIAC populations from which about 100 males and 100 females aged 48 to 56 were examined by CARDIAC health survey. In these Japanese populations 24U T and 24U Na were highly significantly positively associated with each other (p < 0.0001), indicating Japanese had dietary custom to eat seaweed with salty taste. Neither 24U T nor 24U Na were highly significantly positively associated with each other (p < 0.0001). Tooth loss (number of tooth missing) in Maasai and the other subjects was 115 ± 19 mmHg and 130 ± 23 mmHg, respectively. DBP: diastolic blood pressure, T -CHO: total cholesterol.

OXIDATIVE STRESS PROMOTES CORTISOL ACTIVATION OF THE MINERALOCORTICOID RECEPTOR IN THE DEVELOPMENT OF CARDIAC FIBROSIS

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Background: Activation of the mineralocorticoid receptor (MR) in the context of a high salt intake produces vascular inflammation, cardiac fibrosis and heart failure. We have shown that activation of the MR, by endogenous glucocorticoids, when the MR-specificity conferring enzyme 11βHSD2 is inactivated by carbamoyloxime (CBX), is sufficient to produce a similar pathological phenotype. While data show that when plasma aldosterone is low, MR activation is still a key player in the development of heart disease. We hypothesis that in addition to 11βHSD2 activity cellular oxidative stress determines MR activation by endogenous glucocorticoid hormones (cortisol, corticosterone) in the cardiovascular system.

Methods: Rat cardiac myocytes (H9C2 cells), primary vascular smooth muscle cells (VSMC), mouse macrophages (RAW264.7 cells), HepG2 cells (liver carcinoma cell) and kidney fibroblasts (CV-1 cells) were transfected with human MR and an MMTV-luciferase reporter. Transactivation of the MR in response to aldosterone, cortisol and other steroid hormones known to bind the MR in vivo were determined in the presence and absence of 10μM L-BSO or H2O2 to produce oxidative stress or CBX. Blockade of positive responses were achieved by co-administration of spirinolactone. All experiments were normalized to β gal expression and repeated in the absence of transfected hMR to determine specificity of the response for MR. Results: Transactivation of the MR was significantly increased by 0.1 nM-10 nM aldosterone, depending on the cell line. Equivalent transactivation by cortisol was seen at approximately 10-fold higher concentrations in most cell lines. In H9C2 cardiac cells the addition of 10μM L-BSO significantly increased cortisol-mediated transactivation at all concentrations of hormone. L-BSO modestly increased aldosterone mediated transactivation at higher concentrations only. In contrast CBX had no effect on hMR transactivation in H9C2 cells, reflecting the lack of 11βHSD2. Similar responses were found for the RAW cells but not for HepG2 cells or CV-1 cells. MR responses are masked by the markedly higher endogenous GR responses and will be further investigated.

Conclusions: These results show that increasing oxidative stress in increases cortisol activation off the MR in cardiac cells and suggest a mechanism for MR signalling in the presence of low aldosterone levels. The outcome of these studies will have particular relevance for the use of MR antagonists in hypertension, heart disease and coronary artery disease.

ENDOTHELIAL DYSFUNCTION IN MUSCLE RESISTANCE ARTERIES FROM TYPE-2 DIABETIC OBEZE RATS

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The objective of this study was to compare endothelial function in the cremaster artery, a skeletal muscle resistance artery, in male 25-week type-2 diabetic obese Zucker rats and the corresponding 25-week control lean Zucker rats. Endothelium function was assessed using pressure myography, by measurement of the internal diameter of isolated a skeletal muscle resistance artery, in male 25-week type-2 diabetic obese Zucker rats and age-matched lean Zucker rats. Endothelium function was assessed using pressure myography, by measurement of the internal diameter of isolated a skeletal muscle resistance artery, in male 25-week type-2 diabetic obese Zucker rats and age-matched lean Zucker rats. Endothelium function was assessed using pressure myography, by measurement of the internal diameter of isolated a skeletal muscle resistance artery, in male 25-week type-2 diabetic obese Zucker rats and age-matched lean Zucker rats.
BLOOD PRESSURE REGULATION IN GLUCOCORTICOID RECEPTOR KNOCK OUT MICE

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In the present study, we investigated 1) whether ACTH can induce hypertension in glucocorticoid receptor knock out (GRKO) mice, and 2) the effect of mineralocorticoid receptor blockade in GRKO mice.

Male WT and GRKO mice were treated with ACTH (2 mg/kg/day s.c.) or spironolactone (100 mg/kg/day, s.c.) for 1–2 weeks. Blood pressure (BP) and heart rate (HR) were measured using a radio-telemetry system. Urinary Na/K was measured using flame photometry. Blood glucose concentrations and haematocrit were also measured.

Results were expressed as mean ± SEM. Baseline systolic BP (SBP) was higher in male GRKO mice (126 ± 4 mmHg, n = 11) than male wild type (WT) mice (114 ± 2 mmHg, n = 10, P<0.05). There was no difference in baseline diastolic BP (DP), HR and body weight (BW) between male WT and GRKO mice. There was no significant difference in baseline haematocrit, blood glucose and urine Na/K ratio in WT and GRKO mice. ACTH raised SBP in male WT (135 ± 8 mmHg, n = 8; P<0.05) but not in GRKO mice (113 ± 9 mmHg, n = 6). Spironolactone treatment did not alter BP, BW, food and water consumption, urinary output or urine Na/K ratio in male GRKO or WT mice.

In conclusion, basal SBP was higher in GRKO than WT mice; ACTH raised blood pressure in WT but not GRKO mice; and spironolactone did not alter BP in GRKO or WT mice. These data, together with a previous study showing both ACTH and corticosterone are increased in GRKO mice, raises the possibility that increased endogenous ACTH stimulated GC are maximal in this model and may contribute to the increased basal SBP in GRKO mice, possibly via residual fragments of glucocorticoid receptors. Mineralocorticoid receptors do not appear to play a critical role in maintaining blood pressure in glucocorticoid receptor deficient mice.

MATERNAL PROTEIN RESTRICTION DURING PREGNANCY AND LACTATION IN RATS: EFFECTS ON CARDIAC FUNCTION IN ADULTHOOD

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Epidemiological studies have linked intrauterine growth restriction (IUGR) with an increased risk of cardiovascular disease later in life. The aim of this study was to examine the effect of IUGR in rats, due to maternal protein restriction, on cardiac function in adulthood. IUGR was induced in Wistar Kyoto (WKY) dams through administration of a low protein diet (LPD; 8.7% casein) during pregnancy and lactation; the control group received normal protein diet (NPD; 20% casein). In adulthood, cardiac function was assessed in male NPD (N = 11), female NPD (N = 9) and LPD (N = 11) offspring by pressure volumetry using an anesthetized closed chest approach. From our recordings we determined mean arterial pressure (MAP), heart rate (HR) and left ventricular pressure-volume indices under baseline conditions and following dobutamine stimulation (DOB, 2–8 μg/kg/min) and volume loading (lactate solution 20 ml/min per 100 g body mass). Maternal protein restriction led to impaired regulation of cardiac output (CO) during β-adrenergic stimulation in female LPD offspring, whereas regulation of CO in male LPD offspring was not different to NPD controls. We found that heart mass to body mass ratio was not different between groups. Importantly, LPD females maintained a smaller end-diastolic volume (P<0.033) and smaller stroke volume (P<0.003) increase during DOB stimulation, resulting in a significant attenuation of the CO increase (P<0.029). Arterial elastance was also significantly elevated in LPD females (P<0.029), while HR, MAP and maximal and minimal rates of ventricular pressure change were not affected. Changes in indices of contractile function did not differ between groups during volume loading (stretch-dependent mechanisms). In conclusion, these data demonstrate that maternal protein restriction leads to sex specific differences in heart responses to β-adrenergic stimulation in adulthood (female IUGR hearts work harder when challenged).

MODELLING THE IMPACT OF ACHIEVING RISK FACTOR TARGETS ON CVD EVENTS IN A COMMUNITY-BASED STUDY

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Cardiovascular risk scores allow assessment of an individual’s risk of cardiovascular disease. This study aimed to analyse cardiovascular risk scores in the Australian population, and examine the impact of hypothetical prevention strategies on subsequent cardiovascular risk. Individual patient data was collected from the AusDiab (Australian Diabetes, Obesity, and Lifestyle Study) dataset. The patient population was limited to those aged 30–74 as this is the age to which the Framingham risk score applies. An epidemiological model was used to manipulate 5-year Framingham risk scores and observe effects following changes of patient risk factors to target levels (e.g. the cessation of smoking or reaching desired lipid and/or blood pressure levels as specified by ATPIII and JNC VI guidelines respectively). 9790 subjects were included in this study with a mean age of 50.7 ± 11.7 years. Mean risk factor levels were relatively ‘normal’ with approximately 50% of this population having blood pressure and total cholesterol greater than 130 mmHg and 5.2 mmol/L respectively. In this population, changes in blood pressure and total cholesterol provided the greatest impact on reducing cardiovascular risk. As a result, fewer patients require to be treated to prevent one case of non-fatal or fatal CVD. Hence, these are two variables that remain an important target for CV prevention in the general population.
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