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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 circulating levels of IL-6 are altered in ageing. The effect of a single bolus (2mg i.v.) of recombinant human IL-6 on femoral mean arterial blood pressure (MAP), heart rate (HR) and tail vein blood flow (BF) was investigated in young (8–10 wk), old (12 mo), and young rats (WKY) treated with 40 mg/kg/d i.L-NAME for 2 weeks. MAP, HR and BF 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). BF increased (P<0.05) in young rats while a change in MAP was not apparent in the adult 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 (43–52%), an effect that was obliterated following treatment with L-NAME. In young rats, SNP caused significant decrease in MAP that was unchanged by the elevated circulating 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 L-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-dependant. 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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Little evidence exists regarding the per-patient use and costs of cardiovascular medicines in Australia. This study sought to profile annual expenditure on antihypertensive medicines according to vascular bed and gender, using patients with, or at high risk of, atherothrombotic disease enrolled in the Reduction of Atherothrombosis for Continued Health (REACH) registry. 2,873 subjects were recruited into the REACH registry through 273 Australian general practices and community pharmacies between September 2006 to September 2007. For each therapeutic class, a weighted average daily cost (2006 AUD) of antihypertensive medications for the whole study group was $461 (±$5). It was slightly higher for female than male patients ($487 (±$9). The endogenous release of nitric oxide (NO) plays an integral role in the regulation of vascular tone. Recent studies have suggested that nitroxyl (HNO/NO), the one electron reduction product of NO+, may be of physiological importance since it can be produced endogenously and the HNO donor, Angeli’s Salt (AS) dilates conduit and resistance arteries. In addition, HNO can function as a positive inotrope and exhibits distinct pharmacology compared with NO. Together, these findings suggest HNO donors may prove to be a novel treatment for cardiovascular disorders such as angina pectoris. HNO-induced vasorelaxation was not susceptible to the development of tolerance, suggesting that HNO donors may prove to be a novel treatment for cardiovascular disorders such as angina pectoris. HNO-induced vasorelaxation was not susceptible to the development of tolerance, suggesting that HNO donors may prove to be a novel treatment for cardiovascular disorders such as angina pectoris. HNO-induced vasorelaxation was not susceptible to the development of tolerance, suggesting that HNO donors may prove to be a novel treatment for cardiovascular disorders such as angina pectoris.

Acute cardiovascular response to stress: increased dependence on central superoxide production induced by chronic angiotensin II stress

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Chronic activation of the renin angiotensin system leads to hypertension, which may be partly neurogenically mediated. In support of this, we have shown that 2-week subcutaneous infusions of angiotensin II (AngII) lead to activation of specific hypothalamic nuclei known to be involved in regulating sympathetic activity, notably in response to stress. In the present study, we investigated the role of the superoxide radical anion (O2·−) in the cardiovascular responses to stressinduced elevation of plasma AngII and AngII-induced hypertension in rats. The generation of O2·− was assessed using an in vivo lucifer yellow dye technique and the presence of O2·− was confirmed by electron paramagnetic resonance (EPR). Our results demonstrate that the cardiovascular response to stress is increased in the rats treated with AngII and that the elevation in O2·− generation is sensitized in the AngII-treated rats. This suggests that the increased reliance on central superoxide production is a potential mechanism for the increased cardiovascular responsiveness to stress in AngII-treated rats.
we examined whether the function of central pathways regulating sympathetic reflexes (chemoreflex induced by 10% O2 and 3% CO2) and responses to stress (arier, and oscillation, for each 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 (arier) for 1 week. A separate group received both treatments and a control group was included. We also determined the contribution of central sympathetic regulation in the response to stress using the supraventricular tempol. All rabbits were instrumented with an intracerebroventricular (ICV) catheter and an electrode to measure brain 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 RSNA responses to oscillation or arier but the RSNA response to hyperoxia 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 hyperoxia were markedly amplified by chronic stress (P<0.02). Tempol (500 ng/kg/min) had no 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 suproxide production.

**EARLY INTENSIVE BLOOD PRESSURE LOWERING ENHANCES HAEMATOMA RESOLUTION BUT DOES NOT AFFECT PERIHAEMATOMA ODEMA: 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 perihaematoma 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 (≥210 mm Hg) 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 lower) and conventional (target systolic BP 180 mm Hg BP lowering strategy using BP-lowering available intravenous agents). Digital images of baseline and repeat CT (24±5 and 72±3 hours) were performed using standardised techniques and analyzed centrally. Efficacy measures on available repeat images were relative and absolute changes in haematoma (n=256) and perihaematoma oedema volumes (n=270) volumes at 24 and 72 hours. Among patients with ≥3 sequential CT scans, mean SD (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 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 these time points were 3.2% (95% CI 1.0 to 5.4) and 2.7% (95% CI 0.8 to 4.6), respectively (P=0.007). Both groups achieved a large reduction in haematoma volume, time to CT, and haematoma location. Perihaematoma 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 (22.6), 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 perihaematoma 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 visual field loss and optic disc changes. All subjects were excluded if they had any vascular, cardiac, cerebrovascular, or renal disease. We also determined the contribution of central sympathetic regulation in the response to stress using the supraventricular tempol. All rabbits were instrumented with an intracerebroventricular (ICV) catheter and an electrode to measure brain 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 RSNA responses to oscillation or arier but the RSNA response to hyperoxia 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 hyperoxia were markedly amplified by chronic stress (P<0.02). Tempol (500 ng/kg/min) had no 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 suproxide production.

**COMPARATIVE PERFORMANCE OF TRANSIT TIME ALGORITHMS FOR NON-INVASIVE DETERMINATION OF SEGMENTAL AORTIC PWV VELOCITY FROM MRI BLOOD FLUX SIGNALS**

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Background. The availability of blood flow signals during MRI scanning allows measurement of segmental aortic pulse wave velocity (aPWV) from MRI images. However, because of the method of generating the flow velocity curve over several cardiac cycles and the smoothing algorithms affecting the initial onset of the wave, there is considerable variation in estimating pulse transit time. The aim of this study was to assess a range of mathematical algorithms for pulse transit time (PTT) calculation for comparative stability and repeatability. Methods. Seven algorithms (Table) were generated for PTT estimation from MRI aortic flow waveforms (10 subjects, 36±7 years, 4 male). Two measurements were recorded in each subject on different days for repeatability analysis. Outlier PWT results were classed as a “failed” measurement and the success rate calculated. Bland-Altman plots were constructed for each algorithm, and repeatability calculated. Agreement between different methods was calculated using repeated measures analysis. Results. 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 1.9; 2.22 m/s respectively). The intersecting lines algorithm had the best agreement with other pulse transit time algorithms. Conclusions. Whilst agreement between several PWT 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 PWT measurement in general.

**HAEMODYNAMIC 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, 4–6 mm. Simulations were performed with pulsatile and steady pressure and flow (nominal pressure 0.3 mmHg) using normal values of blood density (1.05 g/ml) and viscosity (0.0035 Pa.sec). 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 aneurysm increased with increasing AR. This trend was more pronounced with increasing AR for the parent artery. Results. For constant AR, both the flow rate and maximum velocity in the aneurysm increased with increasing AR. This trend was more pronounced with increasing AR for the 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 ARTERIES 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 mm, n=22; HbA1c: 9.6 ±0.1%, n=11) and control lean Zucker rats (LZR, blood glucose: 9.8 ±0.5 mm, n=21; HbA1c: 6.1 ±0.1%, n=11) due to blood flow to the eye, especially during diastole, in a positive pressure (intraocular) environment. Further work is required to associate these systemic parameters with local ocular blood flow conditions.
20-HYDROXY EICOSATRIENOIC ACID SYNTHESIS IN HUMAN PLATELETS AND NEUTROPHILS AND THE ROLE OF ANGIOTENSIN II AND ENDOTHELIN-1

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Metabolism of arachidonic acid by the cytochrome P450 pathway leads to the formation of 20-hydroxy eicosatrienoic acid (20-HETE) that is a vasoconstrictor in many vessel beds. 20-HETE acts as a second messenger for vasoconstrictor actions of Angiotensin II (Ali) and Endothelin-1 (ET-1) in renal and mesenteric beds in vivo. Previous studies suggested that the 20-HETE is elevated in subjects with features of the metabolic syndrome, a condition associated with high blood pressure and low-grade inflammation. Neutrophils and platelets are integral to the inflammatory process. Both of these cell types can release 20-HETE and have receptors for Ali and ET1. This study examined whether neutrophils and platelets could synthesize 20-HETE in response to All, ET-1 and Calcium ionophore. In this study 14 men aged 53±2.3 yrs, BMI of 24.3±0.7 who were normotensive with SBP=116±3 mmHg and DBP=68±3 mmHg, non-smokers and not taking medication were studied. Platelets were separated from centrifugation and neutrophils were prepared by centrifugation on ficoll-paque and dextran sedimentation. After washing, platelets (1x10⁹) and neutrophils (4.5x10⁹) cells were incubated with saline (control) All or ET-1 (1 μM) for 30 mins at 37°C. After incubation 20-HETE was extracted from the cells, purified and quantitated using gas chromatography mass spectrometry. 20-HETE was present in unstimulated platelets 22.7±2.7 pg. and neutrophils 59.7±7.7 pg. Incubation of platelets and neutrophils with 1 μM of All led to a significant increase in 20-HETE to 42.1±5.4 pg and 79.9±8.3 pg, P=0.05 respectively. Incubation with ET-1 resulted in a significant increase in platelet and neutrophil 20-HETE levels to 47.3±6.9 pg/10⁶ cells and 78.7±8.0 pg/10⁶ cells, P=0.05 respectively. Incubation of platelets and neutrophils with Ca ionophore (2.5 μM I/2) led to platelet 20-HETE levels of 56.1±6.3 pg (P=0.05) and neutrophil 20-HETE levels of 89.2±7.1 pg (P<0.05). The All mediated increase in 20-HETE in platelets and neutrophils could be blocked with PD123293 suggesting the involvement of the AT2 receptor. The All mediated stimulation of 20-HETE could be blocked with BQ787 indicating this process may be mediated via the ET-B receptor. These studies show that platelets and neutrophils can produce 20-HETE in response to All and ET-1. The relevance of this process to clinical situations associated with low-grade inflammation or where All and ET-1 are elevated requires further study.

IMMUNOHISTOCHEMICAL DETECTION OF ADVANCED GLYCACTION 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 leading 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 proceded 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 biopsies from control and diabetic patients. AGEs presence was detected using three different antigodies with three AR protocols, involving different pH and temperatures. The three antibodies were: polyclonal anti-RAGE (1:8000 dilution), monoclonal anti-AGE (6D12, 4 μg/ml) and monoclonal anti-CEL (4F5, 10 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 Protease 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 being by 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 P13-K MEDIATED SIGNALING IN THE NEONATE PRECEDES ADULT PATHOLOGICAL HYPERTROPHY IN THE HYPERTROPHIC HEART RAT (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
programmed' in early life, though the influence of perinatal growth on the development of adult cardiac hypertrophy has not been established. We have previously identified a genetic locus (Lmtr-f) in the spontaneously hypertensive rat (SHR) associated with heart size but not blood pressure, and developed the Hypertrophic Heart Rat (HHR) as a normotensive model of adult primary cardiac hypertrophy. As phenotypic characterization revealed neonatal cardiac growth restriction relative to the control Normal Heart Rat (NHR), this study compared the expression and activation of selected intermediates of growth signalling pathways in young and adult HHR and NHR. Ventricular homogenates were prepared from male adult (12 weeks, n=10) and neonatal (postnatal day 2, n=7) NHR/HHR hearts, and centrifuged (3,000g, 5 mins, 4°C) to recover cytosolic fractions for SDS-PAGE/Western blot analysis (quantified as relative expression units). Despite marked hypertrophy in the adult NHR hearts, no changes were observed (vs NHR) in the phosphorylation status of Akt (1.14±0.09 vs 1.00±0.05, HHR vs NHR, p=ns), GSK3ζ (1.14±0.12 vs 1.00±0.12, p=ns) and ERK1/2 (1.03±0.04 vs 1.00±0.04, p=ns), or in total calcium expression (1.19±0.10 vs 1.00±0.10 p=ns). In contrast, neonatal HHR hearts (growth restricted) exhibited increased Akt phosphorylation (2.31±0.53 vs 0.99±0.07, p=0.029), with decreased phosphorylation of both GSK3ζ (0.46±0.06 vs 1.00±0.17, p<0.001) and ERK1/2 (0.82±0.04 vs 1.00±0.05, p<0.001). Calcium expression was unchanged (0.98±0.09 vs 1.00±0.05, p=ns). These differential activities are consistent with augmented PI3-K mediated 'physiological' growth signalling in the neonatal HHR. These findings indicate that where there is a genetic pre-disposition for hypertrophy, transient growth signalling perturbation in the neonate is observed and may represent an important modeling event in determining the occurrence of adult hypertrophy.

THE EFFECT 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 explored this hypothesis using moderate preterm birth in sheep, a species in which cardiomyocyte maturation closely resembles that in human. 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, and in the left ventricle plus septum. There was, however, a significantly greater ventricular endomysial 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 vasconstriction, are mediated via Ang II type 1 receptors (AT1R). Ang II type 2 receptor (AT2R) stimulation causes a more subtle vasodilator response, which partially counteracts AT 1R-mediated vasoconstriction. However, there are many confounding issues in regards to the 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 nonpeptide selective AT2R agonist, Compound 21, with a Kᵢ value of 0.4 nM for the AT2R and a Kᵢ >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 implanted with catheters for direct blood pressure measurement (left carotid artery) and intravenous 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 10mg/kg/min to 1000mg/kg/min over 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 (50mg/kg/min), or in combination with the AT1R 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 AT2R antagonist (Ro 319 39, 0.5mg/kg) was co-infused, the Compound 21 evoked depressor effect was abolished. Collectively, these results are consistent with those reported using the well-known AT1R agonist, CGP42112, and confirm the original preliminary data (Wan et al, 2004) that Compound 21 evoked BP reductions via AT2R stimulation.


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 alterations 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 dietary eating plan and fish oil supplement (1.8 g per day) on resting cardiovascular function was examined in 32 overweight (BMI 27.7±0.6 kg m⁻²) untrained premenopausal young women (22.0±0.6 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 (DXA, QDR 1500, Hologic). Ventricular blood flow was assessed using phlebography, whereas parasymphathetic 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 aplanation 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 variance 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 cardiac, 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 aplanation tonometry by obtaining augmentation index. FVR was calculated by forearm blood flow (FBF) divided by mean arterial pressure. FBF was measured by Fick’s method using Phlebography with the venous occlusion technique. Blood pressure was monitored continuously using a beat-by-beat tonometry blood pressure sensor (Jentow, Colin Medical). Stroke volume was assessed non-invasively using impedance cardiology. 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 4 weeks of moderate intensity exercise normalized CPBR and arterial stiffness in young individuals with a family history of hypertension.

ADENYLATE CYCLASE ISOFORM mRNA EXPRESSION WITHIN THE ROSTRAL VENTRICALMEDULLA 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...
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 presynaptic vasomotor neurons in the rostral ventrolateral medulla (RVLM) provide tissue-specific control. The aim of this study was to identify functionally distinct effector projections of RVLM presynaptic neurons by axon conduction velocity and catecholamine phenotype. We simultaneously recorded the sympathetic thoracic 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-anasthetised (1.3 g/kg i.p.), paralysed, vagotomised and artificially ventilated Sprague Dawley rats (n = 14). Furthermore, splancnic somatosympathetic responses (SSR) was examined in rats treated with intraperitoneal anti-dopamine-beta-hydroxylase-serotonin (20 mg/kg, s.i., n = 8), a neurotoxin that depleted 60–80% of catecholamine neurons in the RVLM compared to IgG-serotonin control (n = 5). We observed qualitatively different SSR responses in all nerves with interaction terms in the four groups randomised to perindopril-indapamide alone, to glizidace 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). Only one drug 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 < 0.005) 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 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 glizidace 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.

Whole genome survey of copy number variation between the spontaneously hypertensive rat and the Wistar-Kyoto rat

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The spontaneous 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 220x220U array genotypic hybridization (CGH) using a whole-genome array with a 5303kb median probe to detect alterations in DNA copy number between the SHR and normotensive Wistar-Kyoto (WKY) which may possibly play a role in the disease progression in the SHR. Results: The separate effects of routine 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.

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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 benefits with reductions of around one third in nephropathy and close to one fifth in total cardiovascular and renal events, including BP lowering and intensive glucose control are indicated for all patients with type 2 diabetes.

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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, greater rate and more frequent rise in blood pressure. Although the mechanisms underlying this relationship remain unclear, it is possible that an exaggerated response to arousal is an important factor. We therefore determined whether the reactivity of the sympathetic nervous system (SNS) is related to the morning surge in blood pressure. Amplitude, rate pressure product (RPP) values were obtained for overnight sleep and the amount and rate 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 averse stimulus was assessed on a separate day by microencephalographic 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 ± 4.0 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. BPPower and RoR were positively related in total MSNA (ρ = 0.4, P < 0.02) and MSNA amplitude (ρ = -0.5, P < 0.01) observed during the cold pressor test but were not related to the increase in MSNA frequency (ρ < 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.

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 (CW), circumference of thighs (CT), sagittal diameter of abdomen (SDA)), measuring the body fat percentage (BFP), resistive exercise test, measure mean arterial pressure (MAP, heart rate (HR) and locomotor activity. Following exposure, CV variables were recorded at rest and throughout a 1 hr period when a high arousal state 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 BPPower was recorded in BMI mice (102.2 ± 2 mmHg; n = 5) than in BMI mice (86.1 ± 1 mmHg; n = 7). HR levels were also elevated in BMI mice (348 ± 11 bpm) compared with BMI mice (338 ± 5 bpm). During cage swap stress BPPower increased more in BMI mice compared to BMI mice (P < 0.01). Neuronal activation (c-Fos expression) was 33% greater in the paraventricular nucleus of the hypothalamus (P < 0.05) and 27% in the dorsomedial hypothalamus (P < 0.01) in BMI mice (n = 3) compared to BMI mice (n = 3). 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 pressor response to stress suggests that this may be a major underlying central mechanism contributing to the hypertension.

STRESS INDUCED ACTIVATION OF HYPOTHALAMIC BRAIN REGIONS IN GENETICALLY HYPER h TENSIVE MICE

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We have previously shown that the high blood pressure (BPH/2J) strain of “Schlieren” mice also have greater arousal associated rises in blood pressure compared with normotensive mice (BPH/3J). However, it is unclear whether this is due to a neurogenic mechanism or to peripheral vascular and cardiac hypertrophy. Therefore, we examined whether acute aversive stress activates hypothalamic brain regions that may contribute to the hypertension observed in these BPH mice. BPH and BPH mice implanted with telemetry were measured mean arterial pressure (MAP, heart rate (HR) and locomotor activity. Following exposure, CV variables were recorded at rest and throughout a 1 hr period when a high arousal state 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 BPPower was recorded in BMI mice (102.2 ± 2 mmHg; n = 5) than in BMI mice (86.1 ± 1 mmHg; n = 7). HR levels were also elevated in BMI mice (348 ± 11 bpm) compared with BMI mice (338 ± 5 bpm). During cage swap stress BPPower increased more in BMI mice compared to BMI mice (P < 0.01). Neuronal activation (c-Fos expression) was 33% greater in the paraventricular nucleus of the hypothalamus (P < 0.05) and 27% in the dorsomedial hypothalamus (P < 0.01) in BMI mice (n = 3) compared to BMI mice (n = 3). 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 pressor response to stress suggests that this may be a major underlying central mechanism contributing to the hypertension.

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

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Recently, we have shown that pharmacological inhibition of angiotension AT1 receptors in the dorsomedial hypothalamus (DMH) attenuates cardiovascular (CV) stress response. In the present study we determined whether reduced CV reactivity in AT1a receptor 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 exposure, CV variables were recorded at rest and during a 1 hr 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, lower MAP was recorded in AT1a–/– mice (65±2 mmHg; n=7) than 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 (P<0.01) in AT1a–/– mice, as did HR (203±9 vs 121±9 bpm; P<0.01). 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 terminals (P<0.001), paraventricular nucleus, central nucleus of the amygdala, rostral ventrolateral medulla (VMH) (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 VM-PVN (P<0.01) and nucleus of the solitary tract (P<0.05) 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 aversive stress.

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

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The effect of coca 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/d 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 × 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=0.004, 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)**

![Graph showing change in 24 hr MAP (mmHg) for different flavanol doses.](Image)

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

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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 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 I 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 Trait Anxiety (Spielberger’s State and Trait Anxiety Inventory and Beck Depression Inventory II (BDI-II), P<0.02), respectively. Similarly, higher anxiety scores were associated with higher MSNA compared with lower scores, 43\(\pm\)2 vs 34\(\pm\)3 bursts/min (mean\(\pm\)SE; P=0.02), respectively. Similarly, higher anxiety scores were associated with higher MSNA, 43\(\pm\)2 vs 33\(\pm\)3 bursts/min (mean\(\pm\)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\(\pm\)2.2 mmol/L vs 5.6\(\pm\)0.2 mmol/L (mean\(\pm\)SE; P=0.05). Furthermore, higher BDI-II scores tended to be associated with insulin area under the curve, 11484 [9445–12695] 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 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.

**ROLE FOR RENAL ANGIOTENSIN CONVERTING ENZYME 2 IN THE ADAPTATION TO NEPHRON LOSS FOLLOWING SUBTOTAL NEPHRECTOMY 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) surgery. Central rats received vehicle (N=10), and 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 polypria (P<0.001), proteinuria (P<0.01), hypertension (P=0.01), and kidney hypertrophy (P<0.001). In the remnant kidney, autoradiography demonstrated an increased ACE activity binding (P=0.00), whilst cortical and medullary ACE2 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<0.05). ACEI reduced SBP (P<0.01), and inhibited cortical ACE (P<0.001). ACEI was associated with increased cortical and medullary ACE2 activity (P=0.01), and decreased medullary mas 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 its product Ang (1–7) may be useful in the treatment of renal disease.

**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 or in rat tissue engineering chambers (TEC). ASCs in co-culture exhibited a time-dependent increase in cardiac actin mRNA expression (3.68±3.33) times at days 3 and 7, with no further increase at day 14 (34±6). Immunostaining revealed co-expression of cardiac transcription factors, GATA4 and Nkx2.5, and contractile protein cardiac muscle α-actinin (a-actinin) and cardiac myosin heavy chain (cMHC). Furthermore, these cells appeared to contract spontaneously in culture. ASCs were imprinted with hCMs in TEC and constructs compared with implants of ASC or CM alone. After 6 weeks, tissue explants from the chamber were contractile in ASC-CM and CM groups but not in ASC group. Immunohistochemistry showed desmin-positive muscle cells in CM/CM-positive from ASC and ASC-CM group. Further characterization of desmin-positive cells showed staining with a-actinin, cMHC and Cofilin I in cells with CM-like morphology. In conclusion, cell-to-cell interaction was identified as a key inducer for cardiomyogenic differentiation of ASCs. In addition, hASCs transformed in CM and were present in reconstructed cardiac environments. This method was optimized with the contraction of cardiomyocytes and provided a potential cardiac differentiation system for a clinically useful method of cell therapy or tissue engineering.

**COMPREHENSIVE ANALYSIS OF THE ROLE OF ATP-SENSITIVE POTASSIUM CHANNEL GENES, ABC9C AND KCNJB1, 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 KCNJB1) and an ATP-binding cassette (SUR2, encoded by the gene ABC9C) 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 vasomotor 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 ABCC9. 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 (4.7 SD) or fell most on standing (n = 150), mean SBP −12.6 mmHg (4.1 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 VHS 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 renal sympathetic nerve activity (RSNA) depends not just on the level of RSNA per se, but also how the kidney responds to a given level of RSNA. We therefore tested whether mild adiposity alters responsiveness of the kidney to activation of the renal sympathetic nerves. After rabbits were fed a high-fat or control diet for 6 weeks, responses to electrical stimulation of the renal nerves (RNS) were examined under pentobarbital anesthesia. Fat pad mass and body weight were respectively 74% and 6% greater in fat-fed rabbits than controls. Mean arterial pressure and plasma renin activity were similar in conscious fat-fed and control rabbits. RNS in anesthetized rabbits produced frequency-dependent reductions in renal blood flow, cortical and medullary perfusion, glomerular filtration rate, urine flow and sodium excretion and increased renal plasma renin activity overflow. Responses of sodium excretion and medullary perfusion to RNS were significantly enhanced by fat-feeding. For example, 1 Hz RNS reduced sodium excretion by 79.4% in fat-fed rabbits and 46.1% in controls. Two Hz RNS reduced medullary perfusion by 38.11% in fat-fed rabbits and 9.4% in controls. These observations suggest that mild adiposity can augment the antinatriuretic response to activation of the renal nerves by RNS. It is tempting to speculate that this effect is mediated through enhanced neuronal-mediated vasoconstruction in the medullary circulation, since medullary perfusion, which 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 RSNA, but also by increased responsiveness of the kidney to RSNA. 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 (10 (low) or 100 (high) μM/kg/d, i.p.). At both doses, NaHS caused a significant reduction of systolic blood pressure, measured via the tail-cuff method (SBP (μM): control 139±6, high-NaHS 105±5, low-NaHS 98±7, P = 0.001, n = 11). Vasorelaxation to acetylcholine in the abdominal aorta was used as a measure of endothelial function. There was a dose-dependent improvement in the NaHS-treated groups of 10% (low-NaHS) to 20% (high-NaHS, n = 7). NADPH-stimulated superoxide anion generation by aorta, detected by the lucigenin assay, was also reduced by 50% in the high-NaHS treated group (n = 3). Lesion area, determined by oil red O staining, was slightly reduced (7–14%, n = 9) in the high-NaHS treated group. However, about a 4-fold increase in the lesion area compared to control was observed in the high-NaHS treated group. In contrast, levels of the pro-inflammatory cytokine TNF-α were significantly reduced by NaHS treatment. Aortic RXRα levels and reversal of the aortic lesions in the hearts of the vitamin D insufficient offspring (P = 0.04). There was also a significant increase (P = 0.005) 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.

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 cardiac hypertrophy. 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 deplete or vitamin D replete (control) diet for 6 weeks prior to pregnancy, during pregnancy and throughout lactation. Cardiomyocyte number was determined in the fixed left ventricle of offspring (N = 10/group) at 4 weeks of age, using an optical disector/fractator stereotéchnical technique. In other litters, cardiomyocytes were enzymatically isolated from freshly excised left ventricles to determine the proportion of mononucleated and binucleated cardiomyocytes (M = 4 litter/group). 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±0.496×105 cardiomyocytes and 5.568±0.256×105 cardiomyocytes, respectively) and females (6.465±0.646×105 cardiomyocytes and 5.075±0.491×105 cardiomyocytes, respectively). These were accompanied by 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 = 0.005) 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.

HARMONIC COMPLEXITY WITHIN THE RENIN ANGIOTENSIN SYSTEM: ROLE OF THE AG-1(−/−)ACE2/MAS RECEPTOR AXIS

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Characterization of the physiological actions of angiotensin-(1−7) [Ang-(1−7)] provide a new understanding of the role of the RAS in cardiovascular disease by demonstrating that this peptide functioned as an endogenous 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 and RAS. ACE2 can lead to the generation of the biological angiotensin II by mild congeneric hypertensive rats both cardiac ACE2 gene expression and cardiac ACE2 activity are significantly reduced both at baseline and after administration of either isoprenal or losartan. Expanding upon these seminal studies, we now provide evidence for the existence in tissues of an alternate precursor for the formation of Ang II and Ang-(1−7). Prohepernins-2 [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 biochemistry of angiotensin cascade leading to the generation of the biological angiotensin II illuminates the harmonious role by which the renin angiotensin system influences homeostasis.

RECENT FRAMINGHAM OFFSPRING 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 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, 7 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 before the 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 7q22–23, a region of recent coincidence linkage, which is currently under study. Co-twin QTL analysis suggested by us for possible involvement in Familial Hyperaldosteronism type I (FH-I), 8 which is at least five times more common than the glucocorticoid-suppressible Familial Hyperaldosteronism type II (FH-II), for which the mutation is already known and affected subjects are identifiable. Both familial forms are clinically indistinguishable from apparently sporadic cases of primary aldosteronism. Linkage studies in families with FH-II 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 makes the individual, or a general population, of which the individual is a part, more likely to lead to hypertension, given prevailing genetic salt intakes and the "normal" fall in nephron population and GFR with ageing. 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 acute ethanol-exposure on mean arterial pressure (MAP) and renal function in male and female offspring at six months of age. MAP was significantly higher in the ethanol-exposed females, but ethanol-exposed males and significantly lower in ethanol-exposed females in comparison to their controls (Male CON=119±0.002 mmHg, Male ETOH=116±0.2 mmHg; P<0.02). Female CON=108±2.2 mmHg, Female ETOH=119±2.2 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 their controls (Male CON=46.0±0.06 ml/min/gkw, Male ETOH=48.9±0.16 ml/min/gkw, P=0.04. Female CON=42.0±0.09 ml/min/gkw, Female ETOH=42.0±0.08 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=8.43±0.63 ml/min/gkw, Male ETOH=6.45±1.27 ml/min/gkw, P=0.01. Female CON=6.22±0.63 ml/min/gkw, Female ETOH 4.7±0.67 ml/min/gkw P=0.04. Renal vasa recta resistance was significantly higher in the ethanol-exposed females, but ethanol-exposed males were similar to controls (Male CON=1.73±0.19 mmHg/ml/min/gkw, Male ETOH=1.90±0.43 mmHg/ml/min/gkw, P=0.5. Female CON=3.95±0.46 mmHg/ml/min/gkw, Female ETOH=5.95±0.59 mmHg/ml/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 ramipril prevented the development of ACTH-induced hypertension in rats. In the present study, we investigated the effect of losartan, an angiotensin II AT1 receptor blocker, in glucocorticoid-induced hypertension in rats. 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 days starting 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=2031). 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 target blood pressure of grade 1 hypertension (equivalent to 140/80 mm Hg) and hypertensive target (130/80 or 125/75 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 than the ABP value of grade 1 hypertension, possibly through angiotensin II up-regulation of NADPH oxidase.

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 sTudy (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...
8.4 ng/ml). The NPT group (from 108.9/11006 309.5 ng/ml) or 517.1 ng/ml at 2 weeks post treatment) while 308.8 ng/ml at baseline to 2738.8 0.017 and P 0.01 mmHg; PS: 2 0.83, P 0.41). In conclusion, activation of g-protein coupled receptors linked to Gi proteins evokes enhanced responses in SHR versus WKY. In the LVRM the effects of ligand binding to SPCR were abolished by Gi blockade in SHR with no effect on resting levels of BP.

**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 (IEPE; 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 tonometry at rest and post maximal treadmill exercise. Resting PWV was higher in patients with IEPE (10.5 ± 3.1 vs. 8.1 ± 1.7 m/s, P<0.05) but there was no cardiaco-brachial or central SBP between groups (P>0.1). In control patients, IEPE did not significantly change from rest to maximal exercise (11.5 to 10.7; P=0.47), whereas there was an increase of borderline significance (16.3 to 18.8; P=0.06) in patients with IEPE. There was a strong correlation between the change in AIx and the change in E/E' from rest to exercise in patients with IEPE (r=0.83, P<0.001; see figure), but not in controls (r=0.41, P=0.24). In conclusion, exercise-induced changes in arterial haemodynamics are significantly associated with the LV filling pressure response to exercise. Arterial function may be an important target for treating symptoms associated with raised LV filling pressure with exertion.

**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
**051 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 potential agent in an array of animal models of cardiac dysfunction, including diabetes. Diabetic patients exhibit diastolic dysfunction and myocardial remodelling.

In the present study, we hypothesised 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. daily 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, %) were not affected by genotype. Diastolic function was assessed on Doppler echocardiography early/late diastolic filling (E/A ratio). Diastolic dysfunction, cardiac fibrosis (%HW/BW, %) were not affected by genotype. Diastolic function was assessed on Doppler echocardiography early/late diastolic filling (E/A ratio). Diastolic dysfunction, cardiac fibrosis (%HW/BW, %) were not affected by genotype. Diastolic function was assessed on Doppler echocardiography early/late diastolic filling (E/A ratio). Diastolic dysfunction, cardiac fibrosis (%HW/BW, %) were not affected by genotype. Diastolic function was assessed on Doppler echocardiography early/late diastolic filling (E/A ratio). Diastolic dysfunction, cardiac fibrosis (%HW/BW, %) were not affected by genotype. Diastolic function was assessed on Doppler echocardiography early/late diastolic filling (E/A ratio). Diastolic dysfunction, cardiac fibrosis (%HW/BW, %) were not affected by genotype. Diastolic function was assessed on Doppler echocardiography early/late diastolic filling (E/A ratio). Diastolic dysfunction, cardiac fibrosis (%HW/BW, %) were not affected by genotype. Diastolic function was assessed on Doppler echocardiography early/late diastolic filling (E/A ratio). Diastolic dysfunction, cardiac fibrosis (%HW/BW, %) were not affected by genotype. Diastolic function was assessed on Doppler echocardiography early/late diastolic filling (E/A ratio). Diastolic dysfunction, cardiac fibrosis (%HW/BW, %) were not affected by genotype. Diastolic function was assessed on Doppler echocardiography early/late diastolic filling (E/A ratio). Diastolic dysfunction, cardiac fibrosis (%HW/BW, %) were not affected by genotype. Diastolic function was assessed on Doppler echocardiography early/late diastolic filling (E/A ratio). Diastolic dysfunction, cardiac fibrosis (%HW/BW, %) were not affected by genotype. Diastolic function was assessed on Doppler echocardiography early/late diastolic filling (E/A ratio). Diastolic dysfunction, cardiac fibrosis (%HW/BW, %) were not affected by genotype. Diastolic function was assessed on Doppler echocardiography early/late diastolic filling (E/A ratio). Diastolic dysfunction, cardiac fibrosis (%HW/BW, %) were not affected by genotype. Diastolic function was assessed on Doppler echocardiography early/late diastolic filling (E/A ratio). Diastolic dysfunction, cardiac fibrosis (%HW/BW, %) were not affected by genotype. Diastolic function was assessed on Doppler echocardiography early/late diastolic filling (E/A ratio). Diastolic dysfunction, cardiac fibrosis (%HW/BW, %) were not affected by genotype.

Results

<table>
<thead>
<tr>
<th>Glucose</th>
<th>Ntg Sham</th>
<th>Ntg STZ</th>
<th>Tg Sham</th>
<th>Tg STZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.5 ± 1.2</td>
<td>35.5 ± 1.2 *</td>
<td>10.5 ± 1</td>
<td>35.4 ± 1.2 *</td>
<td></td>
</tr>
<tr>
<td>GHB</td>
<td>2.38 ± 0.38</td>
<td>9.04 ± 0.38</td>
<td>2.32 ± 0.33</td>
<td>3.71 ± 0.38</td>
</tr>
<tr>
<td>HW/BW</td>
<td>4.06 ± 0.13</td>
<td>4.11 ± 0.15</td>
<td>5.36 ± 0.13 *</td>
<td>5.25 ± 0.12</td>
</tr>
<tr>
<td>E/A ratio</td>
<td>2.58 ± 0.30</td>
<td>1.49 ± 0.30</td>
<td>3.33 ± 0.26</td>
<td>2.02 ± 0.30 *</td>
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Cardiac fibrosis

| 0.29 ± 0.08 | 0.74 ± 0.08 | 0.33 ± 0.09 | 0.44 ± 0.07 |

*P<0.05 vs. within-genotype sham, #P<0.05 vs. within-treatment Ntg, on two-way ANOVA.

**052 INTRATHORACIC PACAP-38 DIFFERENTIALLY AFFECTS SYMPTOMATIC OUTCOMES**

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Pituitary adenylate cyclase activating polypeptide (PACAP) is an excitatory neuropeptide. PACAP is known to be involved in the central autonomic control of blood pressure, regulated through the rostral ventrolateral medulla. Intrathoracic (IT) administration of PACAP increases splanchic sympathetic nerve activity (sSSNA) and heart rate (HR). These effects indicate a prolonged increase in sympathetic discharge, an important and common factor in essential hypertension. Despite these effects, no change in mean arterial blood pressure (MAP) was observed. In this study, we sought to determine if the lack of MAP response seen upon IT administration of PACAP was caused by differential effects on various sympathetic outflows.

Anesthetised (urethane, 1.5 g/kg, ip) adult male Sprague-Dawley rats (n=31) were vagotomised, ventilated and paralysed. Heart rate was derived from an ECG and a carotid artery catheter was inserted for MAP measurement. End tidal CO2 was monitored, tail blood flow (TBF) was measured with a laser doppler flow probe and the splanchic and cervical sympathetic nerves were recorded as measures of regional sympathetic nerve activity. A catheter was inserted into the IT space at the level of T6. A contralateral carotid artery catheter with 10 μl of PACAP (1 nmol) was placed in with 1 μl PBS, and 10 μl of 1 mM PACAP-38 was injected and washed in with 6 μl PBS. Responses were recorded for 90 mins. Over this period significant increases were seen in HR (37.1 ± 5.3 bpm), sSSNA (89.7 ± 11.1%), cervical sympathetic nerve activity (100.5 ± 35.3%) and CO2 (0.7 ± 0.6 %ET).

However, no significant change was observed in MAP (−12.4 ± 5.4 mmHg) or TBF (54.2 ± 26.5 %). Cause of the increase observed in end tidal CO2 is uncertain. The decreases in MAP occur despite the increase in sympathetic nerve activity in the splanchic and cervical regions. These data suggest that in the vagotomized rat IT PACAP differentially affects sympathetic beds or metabolic processes. The lack of MAP response observed previously may be attributed to this differential effect. The effects of PACAP on sympathetic discharge indicate that dysfunction in the regulation, release or receptors of this peptide may be involved in the etiology of hypertension.
fibrosis. Less is known regarding the role of the angiotensin type 2 receptor (AT2R) in cardiac remodelling. The nitric oxide synthase inhibitor N^G^0-nitro-l-arginine methyl ester (L-NAME) has been used to evoke cardiac remodelling such as increased cardiac fibrosis. Gross et al (2004) determined that L-NAME-treated AT2R-KO mice had greater cardiac perivascular fibrosis than L-NAME-treated wild type mice. These results indirectly suggest that the AT2R is involved in the inhibition of cardiovascular fibrosis. However, the study could not discern the chronic effects of direct stimulation of the AT2R. Thus, the aim of this study was to determine the function of the AT2R in cardiac remodelling via chronic pharmacological stimulation using the AT2R agonist CGP42112.

At 11 weeks old they were treated for 28 days with L-NAME (100mg/kg/day) in drinking water to induce cardiac interstitial fibrosis, while mice were also treated with the selective AT2R agonist CGP42112 (1 μg/kg/min) or saline via osmotic minipumps. Systolic blood pressure was measured via tail cuff before and after treatment. L-NAME caused a significant increase in systolic blood pressure (L-NAME 122 ± 3 mmHg vs. vehicle 96 ± 2 mmHg; P < 0.05). Co-administration with CGP42112 showed a partial reversal of the L-NAME induced increase in systolic blood pressure (113 ± 3 mmHg; P < 0.05 compared to L-NAME alone).

At the conclusion of treatments, hearts were removed to examine cardiac morphology. Cardiac hypertrophy was measured by the ventricular weight to body weight ratio. This ratio was not influenced by L-NAME. However, L-NAME caused a significant increase in cardiac collagen content (L-NAME 4.9 ± 0.2% vs. vehicle 3.3 ± 0.2%, P < 0.05). Interestingly, CGP42112 attenuated the L-NAME induced fibrosis (4.0 ± 0.2% ± P < 0.05 vs. L-NAME).

Therefore, chronic treatment with CGP42112 significantly attenuated the hypertensive and cardiac fibrotic effect of L-NAME treatment in mice. These data suggest that direct chronic AT2R stimulation has both antihypertensive and anti-fibrotic effects, and that these AT2R-evoked effects were at least partially mediated via NO-independent signalling pathways. Gross V. et al (2004) Hypertension; 22: 997-1005.
of 11JPHS2D 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 early 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 cortisol. We determined the effects of a high salt intake on the mechanisms of MR signaling to either aldosterone or cortisol in adult rat cardiomyocytes (H9C2) and in neonatal kidney cells (HEK 293). Transfections were performed with HMR and an 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 oxalate on lipids-specific regulation of MR signaling. These will further our understanding of key mediators responsible for the establishment of cardiovascular disease.

**061**

**EMERGENCE OF AN ENDOTHELIUM-DERIVED HYPERPOLARISING FACTOR (EDHF) COMPONENT IN AORTA FROM THE OBESE 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 9.0±0.2%; n=6) to control normo diabetic male Wistar (Wistar) 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 (0.1–3.0 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 remaining response was abolished by block of small- and intermediate-conductance Ca2+-activated K+ channels with 1 μM TRAM-34 and 1% 2% to 3% 3% preconstriction (n=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 a 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.

**062**

**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 hypertension and cardiac failure; aldosterone is commonly assumed to be responsible for nonepitheilial 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 reparated 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 role of extra cellular glucocorticoids, animals were fed ad libitum at least 1 day before use and provided with standard laboratory chow and 0.9% NaCl drinking solution before inducing ischemia-reperfusion (IR). Cortisol mimicked the action of aldosterone to significantly increase infarct area (IA) (Table) compared to hearts subjected to IR alone, an effect blocked by SP and Tempol, but not RU486. Cortisol (13±1%, N=6) and aldosterone (17±1%, N=6) significantly increased apoptotic index whereas SP blocked cortisol-induced increase in apoptotic index (6±1%, N=7, p<0.05). Perfusion with the MR antagonist SP alone significantly reduced IA below IR alone, suggesting either an excitatory role of endogenous glucocorticoids under conditions of tissue damage or SP affinity for progesterone receptors. Removal of endogenous glucocorticoids by adrenalectomy resulted in a larger IA (52±2%, N=7) and no significant effect of SP (48±1%, N=8). Surprisingly RU486 alone increased IA, but did not block the effect of SP. Cardiac MR are receptors for both glucocorticoids and mineralocorticoids during tissue damage. SP-induced reduction in IA may represent competition with residual endogenous glucocorticoids. The beneficial effect of MR blockade in this model may hold promise for the potential for MR antagonist-eluting stents.

**063**

**EXPOSURE TO PROTEIN RESTRICTION DURING DEVELOPMENT LEADS TO INCREASED WHOLE BODY INSULIN SENSITIVITY IN ADULT MALE AND FEMALE RAT OFFSPRING**

**K Lim,JA Armitage, MJ Black, Department of Anatomy and Developmental Biology Monash University, Victoria**

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 an euglycemic hyperinsulinemic 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 (0.254±0.19 and 6.8946±0.20, males and females respectively) compared to controls (2.7509±0.18 and 4.0369±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 programming of improved glucose metabolism persists into old age is yet to be determined.

**064**

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

**RCY Lin1, JBH Williams1, XJ Du2, GM Xu2, H Kiriazis2, MJ Cowley4, HJ Speirs1**

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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 training; 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 allowing the heart to enlarge in response to physiological stimuli while maintaining normal or enhanced function is the p110 alpha 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. The 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 p110alpha 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-transgenic (Ntg). Dominant negative (dn) PI3K (p110alpha) 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 Ntg, dnPI3K and caPI3K Tg mice to myocardial infarction (MI) for 8 wks. Global mRNA and miRNA expression were identified in Ntg-sham, MI-sham, caPI3K-sham, PI3K-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;
Elevated two-hour glucose post oral glucose tolerance test has been shown to be associated with incident hypertension in the AusDiab study. Isolated clinic hypertension (ICHT) is considered to be an intermediate condition between normotension and hypertension. In a prospective observational study, we have been examining whether AT1A, defined on two separate 24-h ambulatory blood pressure (ABP) recordings, can be better characterized 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. After 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.

**Results:**

<table>
<thead>
<tr>
<th></th>
<th>NT</th>
<th>ICHT</th>
<th>HT</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td>n</td>
<td>17</td>
<td>18</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>55 ± 9</td>
<td>57 ± 8</td>
<td>60 ± 12</td>
<td>NS</td>
</tr>
<tr>
<td>Gender (F)</td>
<td>71%</td>
<td>78%</td>
<td>61%</td>
<td>NS</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>81 ± 14</td>
<td>85 ± 13</td>
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</tr>
<tr>
<td>PWVc (m/sec)</td>
<td>7.8 ± 0.9</td>
<td>8.2 ± 1.2</td>
<td>10.3 ± 2.6</td>
<td>0.008</td>
</tr>
<tr>
<td>Glucose fasting (mmol/L)</td>
<td>4.9 ± 0.4</td>
<td>5.0 ± 0.3</td>
<td>5.0 ± 0.5</td>
<td>NS</td>
</tr>
<tr>
<td>2h glucose (mmol/L)</td>
<td>4.9 ± 1.1</td>
<td>6.4 ± 1.1</td>
<td>6.5 ± 2.0</td>
<td>0.029</td>
</tr>
<tr>
<td>Insulin AUC (units)</td>
<td>4693 ± 1928</td>
<td>5618 ± 2946</td>
<td>7670 ± 5832</td>
<td>0.049</td>
</tr>
</tbody>
</table>

**NS:** Not significant at the 5% level. *p*-value for comparison of IHT group to normotensive group. **p**-value for comparison of HT group to NT group.

**Discussion:**

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

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**Aims:** On the basis of AT1 receptor knockout studies, the AT1 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 AT1 receptor following intracerebroventricular (ICV) administration of AT1 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 h post-stroke (cumulative dose 3.5mg, either alone or in combination with the AT1 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 ischaemic 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 AT1 receptor agonist is neuroprotective independent of any changes in blood pressure. Thus, the current study has shown for the first time that central AT1 receptor stimulation following a cerebral accident is neuroprotective in a conscious rat model of stroke.

**DETRICTARY FRUCTOSE-INDUCED CARDIAC HYPERTROPHY AND OXIDATIVE STRESS IS ASSOCIATED WITH SUPPRESSED MYOCARDIAL SIGNALING 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 (WV) and myocardial production of superoxide (lucigenin chemiluminescence). Phosphorylation states of signalling proteins in myocardial tissue were analysed by western blot. WW was increased by 22% in the fructose fed mice (P<0.05) which was associated with elevated superoxide production (fructose 563± 28 counts/sec/mg vs. control, 489± 11 counts/sec/mg, P = 0.049). Fructose feeding suppressed phosphorylation of Akt and S6 indicating a specific cardiac insulin resistance. Hyperglycaemia (fructose, 14.4±0.6 mmol/L vs. control, 12.1±0.8 mmol/L) and impaired glucose tolerance were observed, but were not associated with hypertension or body weight gain. This 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 anesthesia with intra-aortic catheters that also recorded behavioral movement, 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), ECG electrodes for recording hippocampal theta (5–10 Hz) rhythm. Recordings were carried out in undisturbed conscious unrestrained animals, at ambient temperatures of 22–24°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, a cross correlation −0.9 and no phase difference, heart rate increased by 20±5 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 EGG rhythms were approximately 5 minutes ahead of all the other rhythms. One interpretation of the findings is that rats actively 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 eyelid movement (REM) sleep, Kleitman proposed that a Basic Rest-Activity Cycle (BRAC) persists during 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 Spinaza1, D Roche1, M Braverman1, S McKinley1,3, MC Morel-Kopp1, C Ward1,2, R Bartrop1,2, G Tofler1,2, W Blessing1, Y Ootsuka1, R Menezes1, D Zaretsky2, 1University School of Medicine, Indianapolis, USA, 2School of Biostatistics and Bioinformatics, Indiana University, 3Ramaciotti Center for Gene Function Analysis, University of Sydney, Sydney, Australia, 4Wellcome Trust Sanger Institute, Cambridge, UK

Bereavement is associated with increased risk of cardiovascular disease (CVD), particularly in the early period after the death. We report increased daytime blood pressure and heart rate during the acute early bereavement period. Sixty-two bereaved spouses and partners of deceased persons were recruited in the 2 months after the bereavement. The groups were matched for age, sex and clinical features. They were studied 6 months (6m) and 11 years (13 yrs) after the bereavement period. Daytime systolic (SP) and diastolic pressures (DP) over the 24 h period were measured. The SP/DP load was defined as the percentage of daytime SP/DP readings above 139/80, or below 110/60 mmHg. SP/DP readings routinely exceed these SP/DP load values were increased in the acute early bereavement period, and remained elevated at 13 years. There was a trend for the increased daytime DP load in the bereaved group at 13 years (mean 24%, SD 2). The results provide the first evidence that bereavement is associated with increased daytime blood pressure and heart rate, and new evidence that gender-dependent differences in cardiovascular disease risk are present in the bereavement period. These differences are likely to be multifaceted we now have strong evidence that gender-dependent differences in 24-hour blood pressure are present in bereaved people at least 13 yrs after the bereavement. The increased daytime SP/DP load was associated with a 11% increase in the risk of a cardiovascular event over 13 yrs, with the increased DP load associated with a 26% increase in the risk of a cardiovascular event. The relationship was independent of other cardiovascular risk factors, and remained significant after adjustment for age, smoking, alcohol consumption, and body-mass index. These findings suggest that bereavement may have an important role in the increased CVD risk during bereavement.
having no effect on endothelial adhesion molecules. Therefore HDL affects monocyte – endothelial interactions differentially over time and the interaction with monocytes may be potentially more important in driving the anti-inflammatory action of HDL.

MECHANISMS FOR CHANGE IN AORTIC AUGMENTATION WITH AGE

M Namavarvand1, 2, Adj1, 2, MF O’Reurke1, 2, 2, Department of Cardiology and Vitor Chang Cardiac Research Institute, St. Vincent’s Clinic, Sydney; 3Faculty of Medicine, The University of New South Wales, Sydney; 4Australian School of Advanced Medicine, Macquarie University, Sydney

Augmentation index (AIx) 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 AIx=AP – PP) rise linearly with age. Apparent flattening of AIx over age 60 has been attributed to decreased peripheral wave reflection in older subjects. We sought a purely mathematical explanation of this phenomenon – that two positively sloped linear equations with different intercepts on the y-axis yield a curvilinear flattening of AIx over age 60 has been attributed to decreased peripheral wave reflection in

Estimating absolute risk is considered the best way to identify those most at risk of having a major adverse cardiovascular event (MACE) and therefore who would most likely benefit from intervention. The CVD risk calculators commonly used to estimate risk are based on the 1991 Anderson equation derived from the Framingham cohort study. However no person over 70 years was included in this study. This deficiency needs to be addressed in an ageing society as age is the most important determinant of risk. Method: post hoc analysis of predictive value of the risk equations from Framingham (1991) Anderson and Pocock (for people with hypertension) risk equations for MACE endpoints in the Second Australian National Blood Pressure (ANBP2) study. Analyses: observed and expected 5 year incidence rates, c² goodness of fit tests and ROC curves were used to assess the ability of the Framingham and Pocock equations to predict cardiovascular disease outcomes over 5 years. Results: significant (p<0.05) differences for c² goodness of fit were observed for MI, stroke, CVD morbidity & mortality across age groups and both genders. All ROC curves indicated poor accuracy of the algorithms to predict outcomes CHD & CVD morbidity & mortality, MI, or stroke (these two linear regression lines occurred at critical pressure level (PC) (12±1.4 mmHg). Poor predictors of MACE in the ANBP2 cohort generally overestimating risk. New risk equations for the hypertensive aged are needed. B Neal, 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

BIPHASIC RELATIONSHIP BETWEEN ARTERIAL PRESSURE AND AORTIC PULSE WAVE VELOCITY IN NORMOTENSIVE RATS

K Ng, M Butlin, YY Liu, I Tan, A Avolio, Australian School of Advanced Medicine, Macquarie University, Sydney

Background. Arterial stiffness is an independent predictor of cardiovascular risk. Pulse wave velocity (PWV) is a surrogate of arterial stiffness and is dependent on mean arterial pressure (MAP). The presented study aimed to characterise arterial function over a wide range of MAP by means of quantification of the PWV – MAP relationship.

Methods. Experiments were performed on 6 anaesthetised (Urethane, 1.3 g/kg, ip) Wester Kyoto rats by recording beat-to-beat PWV and MAP using a high fidelity 2.5F catheter with dual pressure sensors 5 cm apart, introduced via the femoral artery and positioned in the descending aorta. Pressure was increased and decreased by intravenous infusion of phenylephrine and sodium nitroprusside respectively. PWV – MAP phase plots were obtained for pressures between 40 and 160 mmHg.

Results. Analysis of PWV-MAP phase plots shows a consistent biphasic pattern with two significantly different slopes in a low pressure (40 – 100 mmHg, 0.8±0.2 m/s/mmHg) and high pressure range (100 – 160 mmHg, 15±0.9 m/s/mmHg, p<0.05). The intersection of these two linear regression lines occurs at critical pressure level (PC) (12±1.4 mmHg). PC was significantly higher than the resting (anaesthetised) MAP (85.5±13.3 mmHg, p<0.05).

Conclusion. Both PC and slopes of the PWV-MAP phase plots represent intrinsic characteristics of arterial distensibility. This may be used to quantify arterial function in the rat aorta with normal and altered arterial wall properties.

CARDIOVASCULAR RESPONSE TO CHRONIC ESTROGEN AND ANGIOTENSIN II INFUSION IN AROMATASE KNOCKOUT MICE

T Nguyen-Huu1, K Denton2, GA Head1, Department of Neuropharmacology, Baker ID Heart and Diabetes Institute, Melbourne; 2Department of Physiology, Monash University, Clayton

Low-dose angiotensin II (Ang II) decreases blood pressure (BP) in female rats, suggesting an estrogen/renn-angiotensin interaction. To confirm the role of estrogen, we investigated the cardiovascular response to Ang II in the estrogen deficient aromatase knockout mouse. Three- to five-month old wildtype (WT; C57BL/J6; n=10) and aromatase knock out (ARKO; n=29) female mice were implanted with telemetry devices. After recovery, mice received either a placebo or extended-release 17β-estradiol (E2) pellet that was inserted transdermally. All WT mice received low doses (2 and 5 mg/kg/min). Mice were allocated into one of four groups, receiving either placebo E2 (ArKo; n=12), low-dose E2 (ArKO+LE2; n=6), mid-dose E2 (ArKO+ME2; n=5), or high-dose E2 (ArKO+HE2; n=6). After two weeks of placebo or E2 treatment, mice were implanted with an osmotic minipump to subcutaneously deliver low-dose Ang II (200 ng/kg/min). 48-hour telemetry recordings of mean arterial pressure (MAP) and heart rate (HR) were conducted after two weeks of placebo or E2 treatment, and after one week of Ang II infusion. MAP was similar between the two placebo-treated strains, though HR was lower in ARKO compared to WT mice (4%; P<0.04). ArKO+LE2 and ArKO+HE2 mice displayed higher diastolic arterial pressure (DAP) compared to ARKO mice (+8% and +7%, respectively; P<0.05), as well as higher HR (+12% and +16%, respectively; P<0.001) from Ang II. Mice had lower DAP compared to placebo-treated ARKO mice (80±2 mmHg compared to 87±2 mmHg; P<0.01). Ang II infusion did not influence BP in WT mice, but increased MAP in placebo-treated ARKO mice (+4.2±2 mmHg; P<0.01), ArKO+ME2 mice (+11.4±5 mmHg; P<0.001) and ArKO+HE2 mice (+18.9±5 mmHg; P<0.001). MAP in ArKO+ME2 and ArKO+HE2 mice displayed a step like increase in response to Ang II infusion, resulting in a difference of 7 mmHg when compared to ArKO mice (P<0.02).

These studies suggest that a lack of estrogen and surprisingly, higher doses of estrogen treatment renders mice susceptible to the pressor effects of Ang II. In contrast, the lower doses were found to have no pressor effects mimicking what is observed in female rats. In conclusion, our findings support the view that the depressor effects of low-dose Ang II are likely to be due to estrogen.
INDEPENDENT IMPACT OF ALBUMINURIA AND KIDNEY FUNCTION ON CARDIOVASCULAR RISK IN PATIENTS WITH TYPE 2 DIABETES: RESULTS FROM THE ADVANCE TRIAL

T Ninomiya1, V Perkovic1, B de Gailan2, S Zoungas3, M Jardine4, A Patel1, A Casa5, B Rinaldi6, M Cooper7, S MacMahon1, J Chalmers5, 1The George Institute for International Health, University of Sydney, 2Daniele Alberti Memorial Centre for Diabetes Complications, Baker Heart Research Institute

Albuminuria 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 diamicroN-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 every halving of baseline UACR and lower baseline GFR levels (both p trend <0.0001). The multivariate-adjusted risks of macrovascular events increased by 31% (95% confidence interval 23–49%) 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 microalbuminuria 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.

Albuminuria and low GFR are independent continuous risk factors for macrovascular outcomes in patients with T2DM.

(ClinicalTrials.gov number, NCT00145925)

KNOWDOWN OF ANGIOTENSINOGEN PROTEIN EXPRESSION IN VITRO

EL O’Callaghan1, WG Thomas2, AM Allen1, 1Department of Physiology, University of Melbourne; 2School of Biomedical Sciences, University of Queensland

The Renin-Angiotensin System (RAS) is defined by its ability to potently regulate blood pressure and fluid homeostasis. This system is made more complex by the existence of several potentially independent tissue RAS, notably in the brain and heart. Current methods are not adequate to resolve independent tissue RAS, notably in the brain and heart. Current methods are not adequate to resolve independent tissue RAS, notably in the brain and heart. Current methods are not adequate to resolve independent tissue RAS, notably in the brain and heart. Current methods are not adequate to resolve independent tissue RAS, notably in the brain and heart. Currently, there is a lack of experimental methods to enable specific knockdown of the angiotensinogen gene in specific brain nuclei. This gives us an unprecedented ability to localise protein knockdown in specific cell types in discrete locations, of particular importance when examining specific brain nuclei.

A novel approach is to knockdown endogenous gene expression of RAS components in specific animal models. In vivo treatment of mice with the angiotensinogen antisense oligonucleotide (Ao-mir) dramatically reduced Ao expression.

These plasmids can be readily converted to enable expression of the mir under the control of independent tissue RAS, notably in the brain and heart. Current methods are not adequate to resolve independent tissue RAS, notably in the brain and heart. Current methods are not adequate to resolve independent tissue RAS, notably in the brain and heart. Current methods are not adequate to resolve independent tissue RAS, notably in the brain and heart. Currently, there is a lack of experimental methods to enable specific knockdown of the angiotensinogen gene in specific brain nuclei. This gives us an unprecedented ability to localise protein knockdown in specific cell types in discrete locations, of particular importance when examining specific brain nuclei.

DIFFERENCES IN TREATMENT PERSISTENCE OVER TIME BETWEEN DIHYDROPYRIDINE ANTIHYPTERTENSIVES

M Ortiz1, G Calcino2, 1Solvay Pharmaceuticals, Sydney, 2Th Connections, Canberra

Treatment compliance with AHT medications is known to be less than ideal. Improved information about patient behaviour could aid the design of interventions to improve treatment compliance. PBS claims data provided by Medicare Australia can be used to identify poor persistence with treatment. Information about treatment persistence (Kaplan-Meier survival) curves is best presented as a graph of the percentage of patients remaining on therapy over time. PBS Claims data for the dihydropyridine (DHP) class of antihypertensive (AHT) can be used to identify non compliant patients. 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 DHP products fall under the General copayment. Initiation means a DHP script after no DHP script for 6 months. A therapeutic episode is considered ceased if there is no script for a DHP product for six consecutive months. Three different groups of patients initiated on a DHP have been considered: ‘AHT naive’, ‘Switch’ and ‘Add on’ patients. Lercanidipine persistence to DHP was superior compared with other DHPs for all three initiation groups (“AHT naive”, “Switch” and “Add on”). However if persistence is re-assessed after the first 6 months, then rates of cessation seem to be similar for all molecules (Figure 1) and for all patient initiation cohorts. This suggests that interventions to improve persistence should be undertaken in the first 6 months post initiation.

DIFFERENCES IN TREATMENT PERSISTENCE WHEN DIHYDROPYRIDINES ARE ADDED TO OTHER ANTIHYPERTENSIVES

G Calcino1, M Ortiz1, 1Th Connections, Canberra, 2Solvay Pharmaceuticals, Sydney

NHF Guide to management of hypertension (2008) states that “based on the best available evidence, the most effective combination we an Angiotensin Converting Enzyme (ACE) inhibitor or an Angiotensin II Receptor Antagonist (ARB) plus a Calcium Channel Blocker (CCB)” If patients don’t take their antihypertensive (AHT) medication, then they won’t lower their BP. Persistence describes how long patients remain on a therapy (until stopping) and information about treatment persistence is best presented as a graph of the percentage of patients remaining on therapy over time. PBS claims data provided by Medicare Australia can be used to identify poor persistence. This analysis is based on all scripts supplied to a one in ten sample

![Figure 1. Rate of DHP molecule cessation after 6 months.](image-url)
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 A2RA while persistence on a DHP was lowest 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 [4–26] 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 amiodipine 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.](image)

**Inferred Mortality Differences Between Dihydropyridine Antihypertensives**

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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 population drawn from de-identified PBS claims between January 2003 and December 2006. Only Concessional patients were included because most DHP 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 (Figure 1) also had a 75% greater inferred mortality (HR 1.88 [1.77 to 2.00]) compared with patients initiated on lercanidipine (Log-Rank statistic [1.91]). Inferred mortality of patients initiated on lercanidipine was consistently lower compared with patients initiated on other DHPs for all three initiation groups ("AHT naïve", "Switch" and "Add on").

![Figure 1. Inferred Mortality by DHP molecule.](image)

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

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PBS Claims data for antihypertensives (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 therapy was assessed for those patients who ceased all AHT treatment for at least 6 months. Almost 7,500 patients, who initiated on a dihydropyridine (DHP), had ceased all AHT treatment in the time window (no AHT script for 6 months), 43% were male and 17% had their DHP initiated by a specialist. The age distribution, of those who ceased all AHT treatment, was < 60 (27.0%), 60 – 69 (17.9%), 70 – 79 (29.0%), and 80+ (26.2%), while the DHP initiation cohorts were "AHT Naıı ve" (51.5%), "Switch" (14.8%) and "Add in" (33.7%). Differences in persistence have been observed between DHP molecules, however there were no differences in the time to restart AHT or proportion restarting AHT treatment. There were differences between DHP initiation cohorts, with around 16–18% of "AHT Naıı ve" or "Switch" patients compared with only 9% of "Add in" patients, restarting any AHT treatment with 12 months of stopping all AHT treatment (Figure 1). Around 30% of "AHT Naıı ve" or "Switch" patients compared with less than 15% of "Add in" patients had restarted any AHT treatment within 3 years. These data suggest that if a patient ceases AHT treatment, then less than one third are likely to restart AHT treatment within 4 years.

![Figure 1. Time to Restart AHT Treatment.](image)

**The Relationship Between Proteinuria and Cardiovascular Disease: A Systematic Review**

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Proteinuria or albuminuria have been reported to be associated with cardiovascular disease, but the consistency and strength of any such relationship overall and for individual endpoints remains uncertain. 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 excluded 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 intervals [CI]: 1.23 – 1.74) 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 [RR] 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**

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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 AT2 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 (Wiemer et al., 2002), to improve endothelial function and to prevent the development of severe hypertension and end-organ damage in SHR (Dent 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 I–7 (24 μg/kg/rat, s.c.) or the angiotensin I–7 mimetic AVE0991 (24 and 120 μg/kg/rat, s.c.). Low dose Ang I–7 was also used with either the AT2 receptor antagonist A-779 (48 μg/kg/hr) or the AT1_R antagonist, PD123319 (10 mg/kg/day).

At the conclusion of treatment, hearts and aorta were removed to examine cardiovascular structure. Indices of cardiac (left ventricular to body weight ratio; cardiomyocyte cross sectional areas) and vascular (medial wall thickness; aortic wall thickness) hypertrophy were not influenced by any drug treatments. However, AVE 0991, Ang I–7 and Candesartan alone all significantly decreased cardiac fibrosis (0.0005% Picrosirius Red). Interestingly, the anti-fibrotic effect of AVE 0991 was reversed by simultaneous treatment with either A779, or PD 123319, suggesting that AT1 receptor may be involved in both the Mas and AT2 receptor in the heart. Therefore, angiotensin I–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 I–7 and/or Mas receptor- and AT1 receptor- mechanisms.

**THE AT,R-MEDIATED DEPRESSION RESPONSE TO ANG II IN FEMALES IS ENDOGENOUS**

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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 normotensive, low dose of Ang II (10 ng/kg/min) which has not altered arterial blood 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 (AT2R). 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 ovariotomy 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 (200 ng/kg/hr, s.c.) or the angiotensin (1–7) mimetic AVE0991 (24 and 120 ng/kg/hr) or the AT2R antagonist, PD123319 (10 mg/kg/day).

As observed previously, MAP significantly decreased in females treated with Ang II (10–2 mmHg), a response that was abolished by ovariotomy (increased 4±2 mmHg) and restored with estradiol replacement (6±2 mmHg). The tidal breath to tidal ratio (T/B) in females was 1.6±0.2 in saline-treated females, whereas in Ang II treated females (T/B) was 2.1±0.2. The gap junction inhibitor that targets connexin 40, 40Gap27 (300 μM), had no effect in arteries from saline treated females, but significantly reduced the NAME/ODQ-resistant response in Ang II treated females (Emax decreased from 27±5 to 9±2, n=8). These findings suggest that although the overall endothelium-dependent relaxation to ACh is unchanged in renal arteries 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 there appears to be a compensatory increase of the EDHF component which may be related to an upregulation of connexin 40-associated gap junction activity.
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 vasodilatation may be related to increased vascular tone. Resting multi-unit sympathetic nerve firing rates were measured at the peroneal nerve by microneurography. Endothelium dependent vasodilatation 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 (NHF). Resting muscle sympathetic nerve activity (MSNA) was higher in EH compared to PFH and NHF (46 ± 18 vs. 25 ± 10 vs. 35 ± 7 bursts/100 heartbeats; 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 PFH compared to NHF (10.7 ± 2.4 vs. 11.1 ± 4.0 vs. 14.8 ± 3.6 ml/min/100m; p < 0.05). In NHF, MSNA correlated significantly with the forearm blood flow response to acetylcholine (r = 0.53; p < 0.05), i.e., the higher the MSNA the higher was 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 vasococontractor effects of muscle sympathetic nerve traffic appear to be counteracted by an adequate response to endothelium derived vasodilator 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.

Systolic Blood Pressure for Guiding the Management of Hypertension (BP Guide): Study Design and Preliminary Results

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Non-invasive estimates of central blood pressure (BP) predict cardiovascular morbidity and mortality independent of brachial BP. However, it is unknown whether central BP may be useful in clinical practice. This study aimed to test the value of central BP as a management tool for physicians treating patients with essential hypertension. Patients with hypertension (n=65; 61±8 years; 54% male) were randomised to 12 months of treatment decisions guided by usual care (UC, n=32; based on clinic, home and 24 hour ambulatory brachial BP) or, in addition, by central BP (CBP, n=33; based on age and gender-specific normal central systolic BP (SBP) values). Titrations (increase, decrease or maintain dose) were provided to each patient’s general practitioner, as well as the patient themselves. Relevant clinical information (e.g. left ventricular [LV] mass, blood biochemistry and symptoms) were considered when making titration recommendations in all patients. Central SBP was estimated by radial tonometry (SphygmoCor 8.0). The primary outcome measures were: 1) change in LV mass (by real time 3D echocardiography), 2) use of medication (as a 5% quality of life. We hypothesized that there will be no significant difference in LV mass between groups (study powered for equivalence). However, it was expected that there will be significantly less use of medication and improved quality of life in the CBP group because more appropriate titration choices will be made to maintain normal central SBP. Baseline LV mass index (CBP, 27.4 ± 5.4 vs. UC, 32.0 ± 6.0 g/m²), brachial SBP (CBP, 129± 14 vs. UC 131± 15 mmHg) and central SBP (CBP, 117± 14 vs. UC 119± 16 mmHg) were similar between groups (P>0.05 for all). However, in the CBP group, 27% (n=9) have received a recommendation to reduce medication, whilst there have been 3% (n=1) in the UC group (P=0.02). Moreover, two CBP patients were recommended to cease antihypertensive medication but maintained normal BP, indicating that they may have been incorrectly diagnosed with hypertension and unnecessarily taking medication based on brachial BP assessments. These preliminary data suggest that therapeutic decisions based on CBP may be different from those based on standard BP. Follow up data and final results (n=312) are expected in 2011.

Phenotype Patterning of Arterial Aging

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The current understanding of arterial aging is based on postmortem analysis of a few individuals over the age of 80 years. In our study we measured arterial aging in 20 adults (age range 19–47) using arterial stiffness imaging. Arterial stiffness decreases progressively from the ascending to the descending aorta (DSc, 1.20±0.16 vs. 0.97±0.15 mmHg·secessary of children was associated with adiposity, fasting glucose, 2 hr glucose, Framingham risk score and traditional cardiovascular risk factor, aged 18 – 80 years of age. Cardiovascular risk factors were also assessed, and the Framingham Risk Score calculated. In age-adjusted analyses, the number of children was associated with adiposity, fasting glucose, 2 hr glucose, Framingham risk score and carotid atherosclerosis in women, but not in men. Multivariate linear regression models indicate that the prevalence of plaques was increased by 15% (95% CI 2 – 29) per child amongst women, and 0% (95% CI 0 – 10) amongst men, after adjustment for age, socioeconomic factors, lifestyle factors and waist circumference. The association between parity and carotid intima-media thickness was similar in younger and older women (PInteraction=0.26).

A higher number of children is associated with increased carotid atherosclerosis in both younger and older women, but not amongst men. These findings indicate that childbearing, but not child-rearing, may be a risk factor for atherosclerosis, and suggest the potential importance of considering the number of children when assessing the level of cardiovascular risk in women.
ALISKIREN DOES NOT CAUSE PARADOXICAL RISES IN BLOOD PRESSURE

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Diuretics, angiotensin converting enzyme inhibitors (ACEIs), angiotensin receptor blockers (ARBs), and aldosterone antagonists (AAs) are all cause-related to plasma renin concentration. High levels of renin have been repeatedly observed with DRLs such as aliskiren (AII). 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 (PBS), or with an active comparator. The frequency of BP rises was evaluated for the pooled monotherapy groups: (1) PBS; (2) ALI 300 mg; (3) high-dose ARB (valsartan 320 mg, valmadison 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 (LPR > 0.1 ng/mL/h). Overall, the frequency of BP rises (% patients) 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 (LPR > 0.1 ng/mL/h).

AII-R APA rises in response to upright posture in AII-R APA, and failure to respond in AII-U APA. As a result, 4 (1303.8 mg/day) in 16 patients with AII-R APA of possible cure. Renin-AII responsiveness is defined as a rise in plasma aldo [PA] following 2h upright posture and also during AII infusion, and it is suggested that this sensitivity to AII may lead to “normal” aldo suppression when renin/AII is basal in plasma aldo [PA]. Radioimmunoassays (RIAs) have lower precision and specificity, but have been performing reliably in a clinical fashion, able to recognise both adequate and inadequate suppression of PA. We have reported the high precision and specificity of our new LC-MS/MS method for plasma aldo [AII], and we have confirmed that RIAs and LC-MS/MS results have been statistically indistinguishable.

SBP or DBP rose by more than 10 mmHg or 5 mmHg respectively, demonstrated a rise in PRA (LPR > 0.1 ng/mL/h). Overall, the frequency of BP rises (% patients) 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 DBP or SBP rise by more than 10 mmHg or 5 mmHg respectively, demonstrated a rise in PRA (LPR > 0.1 ng/mL/h).

During OGTT in obese metabolic syndrome (MetS) subjects. Nineteen insulin resistant (IR) and 12 insulin sensitive (IS) MetS subjects who fulfilled MESP ATP III criteria, participated. The two groups were matched for age, gender and blood pressure (BP). Simultaneous measurements of muscle sympathetic nerve activity (MSNA) by microelectrodeography at the peroneal nerve, whole-body norepinephrine spillover rate, spontaneous cardiac baroreflex sensitivity (bRS), arterial blood flow (QBF) and arterial and heart rate were made at baseline and 14 days of treatment. Both groups had a significant reduction in mean systolic BP (from 156±20 to 109±21 mmHg, P<0.0001) and in heart rate (from 84±12 to 72±14 bpm, P<0.0001). MSNA and norepinephrine spillover rate increased (by 29±7% and 40±13% respectively, P<0.0001; baseline vs 14 days). Weight and body fat mass and insulin resistance index (HOMA-IR) were not changed.

Suppression testing for diagnosis of primary aldosteronism will not lead to overlooking angiotensin-responsive aldosterone-producing adenoma

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Lack of suppressibility of aldosterone (aldo), despite complete renin suppression during salt loading, orally, saluretic infusion or fludrocortisone suppression test (BST), is required to establish a diagnosis of primary aldosteronism (PAS). It has been argued, however, that because of aldosterone sensitivity to renin suppression, such tests might lead to missed diagnosis for Al-Responsive aldosterone-producing adenoma (Al-R APA), which accounts for at least 50% of AAs in our experience, depriving the patient with Al-R APA of possible cure. Renin-AII responsiveness is defined as a rise in plasma aldo [PA] following 2h upright posture and also during AII infusion, and it is suggested that this sensitivity to AII may lead to “normal” aldo suppression when renin/AII is basal in plasma aldo [PA]. Radioimmunoassays (RIAs) have lower precision and specificity, but have been performing reliably in a clinical fashion, able to recognise both adequate and inadequate suppression of PA. We have reported the high precision and specificity of our new LC-MS/MS method for plasma aldo [AII], and we have confirmed that RIAs and LC-MS/MS results have been statistically indistinguishable.

A specific, precise aldosterone mass spectrometry assay to assess clinical performance of a currently available immunoadassay

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We have reported the high precision and specificity of our new LC-MS/MS method for plasma aldosterone. Radioimmunoassays (RAs) have lower precision and specificity, but have been clinically useful in establishing the high incidence of primary aldosteronism (PAS) 4 and in its management. We used the new LC-MS/MS assay to examine whether our current RA (PCP Coat-a-Count) might have led to either over- or under-diagnosis of PAS. Fludrocortisone suppression test (FST) involves saline loading (800ml overnight recombinant) and 1000th (2h) uprightly) and after 3 and 4 days of fludrocortisone and oral salt loading. Day 4 upright PA of >165 pmol/L is diagnostic of PAS. Results by LC-MS/MS for 16 patients with PAS evaluated by RA support the diagnostic performance in each of the three patients undergoing post-op FST following unilateral adrenalectomy for aldosterone-producing adenoma, both RA and LC-MS/MS PAS. Upright PA concentrations were <70 pmol/L confirming cure. Here, no RA had been performing reliably in a clinical fashion, able to recognise both adequate and inadequate suppressibility of PAS. Deming regression analysis comparing results by the two methods, for samples with PA >70 pmol/L (n=49), gave the equation LC-MS/MS = 0.97RA+23. We then stratified into 3 groups: (A) >165 pmol/L (n=11), (B) 150–450 pmol/L (n=43) and (C) <400 pmol/L (n=40). Bland Altman analysis revealed for group (A) a mean bias (RA-LC-MS/MS) of 9.5±11.7 and 95% limits of agreement (LOA) of 43±63 pmol/L for group (B) a mean bias of -7.7 pmol/L and 95% LOA of 125±95 pmol/L for group (C) a mean bias of -20.6±30.6 pmol/L with 95% LOA of 1178±278 pmol/L. For groups (A) and (B) the mean bias was acceptable, but the range of differences between methods was ±50%. For group (C) the mean bias was substantial (RA much lower than LC-MS/MS), and range of differences was substantial.
These data do not support a role for eNOS uncoupling in the genesis of glucocorticoid-induced hypertension.}

In conclusion, sepiapterin does not prevent ACTH- or dexamethasone-induced hypertension.

In summary, the present study was designed to investigate the effect of sepiapterin supplementation on ACTH- and Ang 1–7-induced hypertension in rats. Male Sprague-Dawley rats were treated with sepiapterin (5 mg/kg/day i.p.) or its vehicle (saline (p.)) for 15 days. Saline, Ang (0 μg/kg/day) or sepiapterin (5 mg/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 + Ang (117 ± 3 to 135 ± 3 mmHg, n = 12, P < 0.001) and vehicle + sepiapterin treated rats (from 114 ± 3 to 119 ± 3 mmHg, n = 12, n.s.). Sepiapterin did not change SBP in Ang-treated or sepiapterin-treated rats compared with vehicle treated rats (SBP vehicle + saline 119 ± 3 mmHg, n = 12, vehicle + Ang 135 ± 3 mmHg, n = 12 and vehicle + sepiapterin 133 ± 3 mmHg, n = 13, P > 0.05). Thymus weight was significantly decreased in both Ang and sepiapterin compared with saline treated rats. Sepiapterin did not affect this marker of glucocorticoid activity.

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

**LIGHT EXERCISE REDUCES 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 (minimizing daily activity and the white-coat effect) in congenital heart disease. Forty healthy subjects age 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 radial tonometry 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) and exercise SBP (r = 0.002; P = 0.05). However, radial BP (r = 0.60; P < 0.001) and central SBP (r = 0.48; P = 0.001) 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 BP and 24 ABPM SBP by 2 statistic, which was of borderline significance (r = 0.7; P = 0.056). On multiple linear regression analysis, radial BP but not 24 ABPM SBP, was independently associated (β = 0.6; 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 BP is associated with LV mass index 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. **ANGIOTENSIN 1–7 INCREASES CARDIAC FIBROSIS IN RENAL INJURY**

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Angiogenin converting enzyme (ACE) 2 is a homolog of ACE and is largely confined to kidney and heart. ACE catalyses the cleavage of angiotensin (Ang) II to Ang 1–7, thereby limiting vasoconstrictor actions of Ang II, and promoting the progression of Ang 1–7, which has been shown to have anti-fibrotic effects in a number of models. We have previously shown that cardiac fibrosis is present in renal failure and is associated with increased cardiac ACE2, which may be a compensatory response to increased Ang 1–7. However, the effects of Ang 1–7 in the setting of renal injury have not been investigated. The aim of this study was to assess the effects of Ang 1–7 infusion on cardiac fibrosis in a rat model of renal injury. Sprague-Dawley rats had either sham (control) or subtotal nephrectomy (Snx) surgery. All control rats received vehicle (n = 8), and Snx rats received either vehicle or an ACE inhibitor (Avas 1 mg/kg/day) by daily gavage or Ang 1–7 (24 μg/kg/day) via subcutaneous minipump for 10 days after surgery (n = 8/group). Evidence of renal impairment by Snx was shown by elevated 24 hour urine volume (P < 0.01) and reduced creatinine clearance (P < 0.01). At 10 days, Snx also led to an increase in systolic blood pressure (SBP; P = 0.001), which was reduced with an ACE (P < 0.01) but unchanged with Ang 1–7. Heart and left ventricle/body weight ratios were increased in the Snx group compared to control animals (P < 0.001) and decreased with an ACE (P < 0.01). Snx was associated with cardiac injury as indicated by increased cardiac fibrosis using collagen staining (P < 0.001) and an increase in ACE binding was also observed (P < 0.01). Interestingly, heart and left ventricle/body weight ratios (P < 0.005), cardiac fibrosis (P < 0.005) and ACE binding (P < 0.001) were all further increased with administration of Ang 1–7 compared to the vehicle treated Snx group. These data suggest cardiac ACE is increased in an attempt to break down the anti-fibrotic Ang 1–7 thereby resulting in cardiac fibrosis. Further studies are required to understand the mechanism of these unexpected effects.

**KYNURENINE IS A NOVEL ENDOTHELIUM-DERIVED VASCULAR RELAXING FACTOR PRODUCED DURING INFLAMMATION**

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Control of blood vessel tone is central to vascular homeostasis. Here, we report kynurenine (Kyn) as a novel endothelium-derived vascular relaxing factor formed from tryptophan by the enzyme indoleamine-2,3-dioxygenase (IDO) during systemic inflammation. Experimental systemic inflammation, including cerebral malaria (infected with Plasmodium berghei ANKA), non-cerebral malaria (infected with Plasmodium berghei K17), and sepsis, caused endothelial expression of IDO, resulting in decreased platelet aggregation, increased Kyn, and systemic hypertension. Pharmacological inhibition of IDO increased blood pressure in infected mice, but not in uninfected animals or in mice deficient in IDO or interleukin-10, which is required for IDO induction. Tryptophan dilitated pre-constricted porcine coronary arteries only if active IDO and an intact endothelium were present. Kyn dose-response with resting brachial SBP (r = 0.31; P = 0.06), radial SBP (r = 0.03; P = 0.8), and central SBP (r = 0.02; P = 0.05). We conclude that exercise radial BP is associated with LV mass index 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.

**INHIBITION OF ANGIOTENSIN II TYPE 1 RECEPTOR EXCLUSION USING RNA INTERFERENCE TO REDUCE BLOOD PRESSURE IN THE SPONTANEOUSLY HYPERTENSIVE RAT**

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We examined a new paradigm for the control of high blood pressure using RNA interference (RNAi) gene technology to inhibit the expression of angiotensin II type 1
RNaseA 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 euthanized 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 RNAi plasmid (n=6), a control plasmid containing green fluorescent protein (GFP, n=6) or vehicle treated controls, was injected into the liver via vein injection. The animals were followed for a further 8 weeks. Additional animals were treated with RNAi plasmid or saline and euthanized 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 and 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-HYDROXYEICOSATETRAENIC ACID GENERATION

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20-hydroxyeicosatetraenonic acid (20-HETE) is a major product of cytochrome P450 (CYP450) catalysed metabolism of arachidonic acid. 20-HETE has vasoactive and autocrine 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 synthesis at 50 μM Enteroaactone and enteroaolin, 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).

In conclusion, the TG as well as body fat% were decreased by taking well-balanced lunch once a day, over 4 weeks. Since our previous trial, “well-balanced lunch once a day” was proven to reduce arterial stiffness (PWVc) and brachial artery flow-mediated dilation (FMD) in pre-diabetes and type 2 diabetes mellitus (T2DM). PWVc significantly correlated to fasting glucose (BGL) and HbA1c (r = 0.31, p<0.003 and r = 0.35, p<0.0001 respectively). After adjusting for age, BMI, MAP, cholesterol and fasting insulin, there was no significant difference in PWVc and HbA1c remained significant (r = 0.37, p<0.06). Multiple regression analysis including above factors and treatment with oral hypoglycaemic agents demonstrated a significant relationship between PWVc and BGL (β = 0.19, p<0.01) and between PWVc and HbA1c (β = 0.28, p<0.04). Correlation between PWVc with BMI and HbA1c remained (r = 0.27, p<0.03 and r = 0.37, p<0.01 respectively) was not significant after adjustment. PWVc and FMD were not significantly correlated with INS. Arterial disease in pre-diabetes and T2DM is characterised by a progressive increase in PWVc. This appears to be mediated by hyperglycaemia rather than hyperinsulinaemia.

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 businesspeople 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 (control, soy rich lunch, fish rich lunch). The intervention lasted 5 weeks, and all lunches contained the same energy content (600 Kcal), same amount of protein and fat and 3.5 g of salt. Health survey including blood sampling and 24-hour urine collection was carried out prior to and after the intervention. Triglycerides: at baseline, the TG of S group was significantly decreased from the baseline (P<0.01), and significant inter-group differences were observed between C and S groups (P<0.05). 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 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 diets 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.

HYPERGLYCAEMIA RATHER THAN HYPERINSULINAEMIA IS IMPLICATED IN ARTERIAL STIFFNESS AND ENDOTHELIAL DYSFUNCTION IN TYPE 2 DIABETES MELLITUS

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Increased central arterial stiffness and endothelial dysfunction are independent risk factors for cardiovascular events. Hyperglycaemia and hyperinsulinaemia as causes for vascular dysfunction in pre-diabetes and type 2 diabetes mellitus (T2DM) were explored. Non-smokers, aged 50-75 with normal renal function were recruited (n=101). Fasting blood samples were collected. Supine mean arterial blood pressure (MAP), aorto-renaluminal pulse waveform (PWVc) and brachial artery flow-mediated dilation (FMD) were measured. A 75 g oral glucose tolerance test defined hyperglycaemic state. Data are presented as mean±SE with analysis using pearson correlation, one-way ANOVA and multiple regression. Mean age of subjects was 63.5 years. PWVc increased significantly from control to pre-diabetes and T2DM. PWc significantly correlated to fasting glucose (BGL) and HbA1c (r = 0.31, p<0.003 and r = 0.35, p<0.0001 respectively). After adjusting for age, BMI, MAP, cholesterol and fasting insulin, partial correlations for PWVc and HbA1c remained significant (r = 0.37, p<0.06). Multiple regression analysis including above factors and treatment with oral hypoglycaemic agents demonstrated a significant relationship between PWVc and BGL (β = 0.19, p<0.01) and between PWVc and HbA1c (β = 0.28, p<0.04). Correlation between FMD with BGL and HbA1c (r = 0.27, p<0.03 and r = 0.37, p<0.01 respectively) was not significant after adjustment. PWVc and FMD were not significantly correlated with INS. Arterial disease in pre-diabetes and T2DM is characterised by a progressive increase in PWVc. This appears to be mediated by hyperglycaemia rather than hyperinsulinaemia.
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 seventy-three 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-h urine collection and the examination of dental status and periodontal status. Results: Systolic BP (SBP) in Maasai and the other subjects was 115 ± 19 mmHg and 130 ± 23 mmHg, respectively. Diastolic BP (DBP) averaged 68 ± 13 mmHg, 75 ± 15 mmHg, respectively. The average of SBP and DBP was significantly lower in Maasai than the other subjects (p < 0.0001). 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 utilization of clean teeth and dairy intake of traditional yogurt recently known to have immunopotentiating effect.

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 (11)β-hydroxysteroid dehydrogenase 2 (11βHSD2) is inactivated by carbonyl oxide (CBX), is sufficient to produce a similar pathological phenotype. Using mouse data we show that when plasma aldosterone is low, MR activation is still a key player in the development of heart disease. We hypothesize 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 spironolactone. All experiments were normalized to β-gal expression and repeated in the absence of transfected MR to determine specificity of the response for MR. Results: Transactivation of the MR was significantly increased by 0.1 µM 11β-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 MR 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.

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 salt 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 is 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 induced hypertension rats show that the effect of T on cardiovascular function was only one factor that might contribute to the prevention of both cardiovascular diseases. Taurine plays a key role in the cardiovascular system, triggering the activation of the MR, by endogenous glucocorticoids, when the MR-specificity conferring enzyme (11)β-hydroxysteroid dehydrogenase 2 (11βHSD2) is inactivated by carbenoxolone (CBX), is sufficient to produce a similar pathological phenotype. Using mouse data we show that when plasma aldosterone is low, MR activation is still a key player in the development of heart disease. We hypothesize that in addition to 11βHSD2 activity cellular oxidative stress determines MR activation by endogenous glucocorticoid hormones (cortisol, corticosterone) in the cardiovascular system.

ENDOTHELIAL DYSFUNCTION IN MUSCLE RESISTANCE ARTERIES FROM TYPE-2 DIABETIC OBSESE ZUCKER 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 segments of cannulated cremaster arteries suspended in MOPS (3-(N-morpholino)propanesulfonic acid)-modified Krebs buffer, and maintained at a physiological pressure of 95±5 mmHg. The arterial segments were excised from male Zucker rats before 25 weeks of age and considered as controls. Endothelium-dependent vasodilation to acetylcholine (ACh) was determined in isolated cremaster arteries taken from male Zucker rats. To evaluate the role of increased oxidative stress, rats were treated with L-NAME (100µM), which significantly impaired the response to ACh (ANOVA, P<0.05) in both obese and lean Zucker rats. Significantly the response was abolished by inhibition of the nitric oxide (NO) pathway using L-arginine (arginine/NAME, 100µM, and 1H-[1,2]oxadiazole[4,3-d]pyrazin-1-amine (ODQ, 1µM). These results indicate that there is endothelial dysfunction in cremaster muscle resistance arteries from type-2 diabetic rats, and that ACh-induced vasodilation in lean and obese Zucker rats is mediated by two vasodilator components, namely EDHF and NO.

<table>
<thead>
<tr>
<th>Group</th>
<th>SBP (mmHg)</th>
<th>DBP (mmHg)</th>
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<td>(M )</td>
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<td>(PF )</td>
<td>121.8 ± 2.9 (43)</td>
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</table>

**p < 0.05, **p < 0.01
MATERIAL 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=10) 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), 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).
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