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**Abstracts From the 35th Annual Scientific Meeting of the HBPRCA and the 39th Annual Scientific Meeting of the AAS**

**H-001**

**EXCESS PRENATAL CORTICOSTERONE EXPOSURE PROGRAMS HYPOTENSION, VASCULAR REMODELLING AND ALTERS THE RESPONSE TO RESTRAINT STRESS IN ADULT MALE MICE**

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**Background:** Exposure to excess glucocorticoids in utero is known to program susceptibility to cardiovascular disease in later life. Endogenous levels of glucocorticoids are increased during periods of stress. The long-term cardiovascular implications for the offspring of women who suffer from stress during pregnancy are, however, poorly understood.

**Aim:** To examine the direct effects of prenatal corticosterone (CORT) exposure on cardiovascular and renal outcomes in adult offspring.

**Methods:** Pregnant mothers received CORT (33 g/kg/h) for 60 h from embryonic day 12.5 and allowed to litter naturally. Nephron number was determined in the offspring at postnatal day 30, and radiotelemetry was used to measure blood pressure and heart rate (both basal and during restraint stress) at 12 months. Pressurized myography was used to assess vascular function, structure and mechanics.

**Results:** Excess prenatal CORT reduced nephron endowment by 33% and 20% in male and female offspring (respectively) compared to untreated controls (n=6–7; P<0.05). At 12 months, CORT-exposed male offspring were hypertensive: MAP 104.3±11.0 vs 115.6±5.5 mmHg in untreated controls (n=8–9; P<0.05). These mice also showed a blunted tachycardic response to restraint stress. Power spectral density analysis of heart rate suggested that this may be due to an altered vagal/sympathetic balance. Prenatal CORT reduced activity levels of female offspring at night, but did not alter any cardiovascular parameters. In male offspring (but not females), prenatal CORT caused inward remodeling of mesenteric arteries (luminal diameter = 2027 m vs 2499 m in controls) without affecting vascular reactivity or the elasticity of vascular wall components.

**Conclusion:** Prenatal CORT exposure programs hypotension in male offspring, despite the reduced nephron number and inward vascular remodeling in these animals. In contrast, CORT-exposed female offspring have reduced nocturnal activity levels (possibly indicating anxiety-like behaviour) and fewer nephrons, with no other cardiovascular alterations. Together, these data demonstrate that excess prenatal CORT causes long-term alterations to the renal and cardiovascular systems, as well as central control mechanisms.

**H-002**

**THE INTRARENAL RENIN-ANGIOTENSIN SYSTEM (RAS) IN HUMAN PREGNANCY**

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**Background:** Components of the renin-angiotensin system (RAS) are found in the renal tubules. Angiotensin II plays an important role in tubular sodium reabsorption. In human pregnancy, increased salt reabsorption is required to maintain maternal blood volume. Failure to activate the IRAS in pregnancy may reduce maternal blood volume and ultimately predispose to intrauterine growth retardation and/or preeclampsia.

**Aim:** To determine whether alterations occur in levels of other IRAS components.

**Methods:** Pregestational diabetic pregnancy was treated with metformin (33 g/kg/h) for 60 h from embryonic day 12.5 and allowed to litter naturally. Nephron number was determined in the offspring at postnatal day 30, and radiotelemetry was used to measure blood pressure and heart rate (both basal and during restraint stress) at 12 months. Pressurized myography was used to assess vascular function, structure and mechanics.

**Results:** Excess prenatal CORT reduced nephron endowment by 33% and 20% in male and female offspring (respectively) compared to untreated controls (n=6–7; P<0.05). At 12 months, CORT-exposed male offspring were hypertensive: MAP 104.3±11.0 vs 115.6±5.5 mmHg in untreated controls (n=8–9; P<0.05). These mice also showed a blunted tachycardic response to restraint stress. Power spectral density analysis of heart rate suggested that this may be due to an altered vagal/sympathetic balance. Prenatal CORT reduced activity levels of female offspring at night, but did not alter any cardiovascular parameters. In male offspring (but not females), prenatal CORT caused inward remodeling of mesenteric arteries (luminal diameter = 2027 m vs 2499 m in controls) without affecting vascular reactivity or the elasticity of vascular wall components.

**Conclusion:** Prenatal CORT exposure programs hypotension in male offspring, despite the reduced nephron number and inward vascular remodeling in these animals. In contrast, CORT-exposed female offspring have reduced nocturnal activity levels (possibly indicating anxiety-like behaviour) and fewer nephrons, with no other cardiovascular alterations. Together, these data demonstrate that excess prenatal CORT causes long-term alterations to the renal and cardiovascular systems, as well as central control mechanisms.

**H-003**

**MATERNAL STRESS DURING PREGNANCY PROGRAMS NEPHRON DEFICITS AND GENDER SPECIFIC HYPERTENSION IN SECOND GENERATION OFFSPRING**

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**Background:** Being born small increases the risk of cardiovascular disease, especially in males. Evidence suggests that disease risks are not limited to the first, directly exposed generation (F1) but can be transmitted to the next generation (F2). Stress during pregnancy adversely impacts fetal development.

**Aim:** To characterize nephron endowment and blood pressure in F2 offspring born to normally grown and growth restricted (F1) mothers and to further assess the impact of maternal stress during late pregnancy.

**Methods:** Male gestation rat uteroplacental insufficiency was induced by bilateral uterine vessel ligation (restricted) or sham (control) surgery in F0 females. F1 females were mated and allocated to unrestricted or late pregnancy stressed (24 h metabolic cage, tail cuff blood pressure, glucose tolerance test) groups. F2 body weights (birth to 12 months), nephron number (day 35) and blood pressure (6, 9 and 12 months) were measured.

**Results:** F2 offspring born to mothers exposed to maternal stress had reduced weight at birth (~5, P<0.05), but no differences thereafter. There were no differences in heart, left ventricle or kidney weights. Nephron number was reduced in offspring exposed to maternal stress with males more affected (~20%; P<0.05) than females (~14%; P<0.05), with no effect on renal function. Restricted unstimulated males had high blood pressure at 6 and 12 months compared to control unstimulated (+7 mmHg; P<0.05). Control male offspring exposed to maternal stress during pregnancy had elevated blood pressure at 6, 9 and 12 months compared to control unstimulated (+10–13 mmHg; P<0.05). This maternal stress during pregnancy response in control males resulted in an elevated blood pressure that was not different to the high blood pressure of male offspring to unstimulated mothers who were born small.

**Conclusion:** Maternal stress in pregnancy reduced nephron endowment regardless of maternal birth weight. Mothers, born of normal birth weight, but exposed to stress during pregnancy, produced males that developed adult hypertension to a similar extent to that of males whose mothers were born small but not exposed to stress. Maternal stress in pregnancy has profound cardio-renal effects on the next generation offspring regardless of whether the mother was born small or born of normal birth weight.

**H-004**

**ALTERATIONS IN RENAL FUNCTION OCCUR PRIOR TO THE INCREASE IN ARTERIAL PRESSURE IN RAT OFFSPRING FOLLOWING SHORT-TERM EXPOSURE TO MATERNAL CORTICOSTERONE**

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**Background:** An acute exposure of the fetus to elevated levels of maternal corticosterone (CORT, natural glucocorticoid in rodents) results in reduced nephron number and hypertension in rat offspring in adulthood. At birth, each nephron must rapidly adapt to take over the role of maintaining extracellular fluid (ECF) homeostasis from the placenta. In a kidney with fewer nephrons, hypotrophy of the glomerulus and tubule occurs to enable the remnant nephrons to handle a larger share of ECF. We hypothesize that these structural adaptations are accompanied by adaptations in glomerular and tubular function, which in the short-term will normalize renal function, but in the long-term will increase the risk of renal and cardiovascular disease.

**Aim:** To examine the changes in arterial pressure, renal function and tubuloglomerular feedback (TGF) during the postnatal period in rat offspring exposed to elevated levels of maternal CORT in utero.

**Methods:** In male offspring (n=8; Sprague-Dawley rats) of mothers treated with CORT 0.8 mg/kg/day i.p. bid or vehicle (VEH; 0.2 ml/kg/day) on days 14 and 15 of gestation (21 days), mean arterial pressure (MAP) was recorded via radio-telemetry from 3–6 weeks of age. TGF was assessed via renal micropuncture at 3 and 10 weeks of age under basal conditions and during intra-tubular nitric oxide (NO) blockade with L-NNAME.

**Results:** MAP was not significantly different between the offspring of VEH and CORT groups at a postnatal age of 25 days. MAP increased with age (P<0.002) in both groups. However, the increase in MAP was enhanced in the offspring of the CORT-treated group (P<0.001). There was a leftward shift of the TGF response curve in offspring of the CORT group at 3 weeks of age, which was associated with a reduced contribution of NO.
**Conclusion:** In offspring with a congenital nephron deficit induced by maternal glucocorticoid administration, TGF is sensitized at a postnatal age of 3 weeks to allow increased expression of sodium and fluid and so reducing single nephron glomerular filtration rate. These changes occur prior to the increase in arterial pressure suggesting that the alterations in TGF early in life may be a potential mechanism that could drive the development of hypertension later in life.

**REFERENCES**

Cai X, Kett MM

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**Background:** Obesity is associated with complications during pregnancy, including hypertensive disorders of pregnancy, and increased risk of cardiovascular and renal disease later in life. Research into the impact of obesity on the normal cardiovascular and renal adaptations that occur during pregnancy.

**Aims:** To measure mean arterial pressure (MAP), heart rate (HR) and renal function across pregnancy in a mouse model of diet-induced obesity.

**Methods:** Four-week old female C57BL6J mice were placed on control (7% fat, 3.85 kCal/g) or high fat (HF; 23.5% fat, 4.54 kCal/g) chow for 10 weeks. Baseline glucose tolerance (n=10, 16), MAP and HR (radio-telemetry; n=12, 16), and 24-hour albumin excretion (n=6, 8) were measured. MAP and HR were measured throughout gestation and albumin excretion was reassessed at gestational age (GA) 13.

**Results:** After 10 weeks of the diet, body weight of HF mice was 44% greater than control mice (52.7 ± 0.9 vs 22.7 ± 0.4 g; P<0.001). Obese mice were hypertrophying (PI 9.1 ± 0.4 vs 7.6 ± 0.5 mmol/L; P<0.05), glucose intolerant (716 ± 43 vs 490 ± 41 mmHg; P<0.001), had elevated 24-hour MAP (105.7 ± 1.3 vs 98.1 ± 1.4 mmHg; P<0.001) and HR (548 ± 8 vs 492 ± 9 bpm; P<0.001) compared to control mice. MAP and HR of obese mice remained elevated compared to control mice throughout pregnancy (P<0.001). The adaptations of MAP and HR were apparent in male mice during first half of pregnancy, including the mid-gestation fall in MAP at GA 9.4 were consistent in timing and magnitude with control mice. However, the rise in MAP in the later phase of pregnancy, near term, was blunt compared to control mice (P<0.05, 0.001, 0.001). The marked rise in HR observed in control mice during the second half of pregnancy was also significantly blunted in obesity mice such that HR was no longer different between the two groups from GA 11 (P<0.05). Both groups had increased albuminuria at GA 13 (P<0.001). This increase was, however, –3-fold greater in obese mice (P<0.01).

**Conclusion:** Obesity results in higher MAP and HR that persists throughout pregnancy. Obese mice demonstrated, however, a blunted increase in HR and to a lesser extent MAP in the later stages of pregnancy. As the high cardiac output (CO) in the second half of pregnancy is dependent on an increase in HR, our data suggests that obesity may limit the increase in CO late in pregnancy, increasing the risk of maternal and fetal complications. Further, pregnancy exacerbates albuminuria in obese females, suggesting that obesity may increase the risk of renal complications during pregnancy and lead to renal disease in later life.
indistinguishable from WT. Although the functional substitutions are remote from the selectivity filter, they (i) influence protein folding and rectification, (ii) the selectivity of the KCNJ5 channel, and (iii) Ang II-induced aldosterone release from the H295R cell line. Clinically, the rs7102584 SNP was associated with less florid PA.

Conclusion: These data suggest that germ-line variations in the KCNJ5 gene play a role in the common sporadic as well as in the rare syndromic forms of PA. Further studies are needed to confirm these findings and explore roles of other germ-line variants in KCNJ5 in various forms of PA.

REGULATION OF ALDOSTERONE SYNTHASE (CYP11B2) BY miR-125a-5p AND miR-125b
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Background: Essential hypertension is known to have a large genetic component. Single nucleotide polymorphisms (SNPs) in the gene CYP11B2, which encodes the aldosterone synthase enzyme, are associated with excess aldosterone production and hypertension. Currently the causative mechanism remains elusive. We propose a regulatory role for microRNA (miRNA). miRNAs are small, endogenous RNA molecules that negatively regulate mRNA abundance by binding to the 3’-untranslated region (3’UTR) of their targets. Augmented miRNA expression and function have been implicated in various pathologies.

Aim: To investigate whether miRNAs expressed in the human adrenal gland regulate CYP11B2 mRNA and, to determine if the impact of CYP11B2 SNPs on miRNA function.

Methods: miRNAs predicted to target CYP11B2 were identified using four bioinformatics algorithms, then cross-referenced with miRNA microarray expression data from normal human adrenal glands and aldosterone-producing adenomas (APA). Four miRNAs were tested in vitro for regulation of CYP11B2 mRNA.

Results: Microarray and bioinformatic data identified four adrenal miRNAs (miR-125a-5p, miR-125b, miR-134 and miR-495a) having putative binding sites in CYP11B2 mRNA. All four miRNAs were tested using a CYP11B2 3’UTR reporter plasmid that was co-transfected alongside a synthetic miRNA (pre-miR) or miRNA inhibitor (anti-miR). Both miR-125a-5p and miR-125b pre-miRs significantly decreased luciferase activity when over-expressed. Conversely, their anti-miRs significantly increased luciferase activity. Furthermore, CYP11B2 mRNA levels were significantly altered in H295R adrenal cortical cells following modulation of miR-125a-5p and miR-125b levels, consistent with canonical miRNA binding and repression. Modification of miR-495a and miR-134 levels did not alter luciferase activity significantly. Comparison of miRNA expression levels showed that miR-125a-5p and miR-134 are significantly down regulated in APAs. Mapping of CYP11B2 intrinsic and 3’UTR SNPs indicated that genetic variation is unlikely to have an impact on miRNA expression or function.

Conclusion: We have identified two miRNAs, miR-125a-5p and miR-134, that exhibit altered expression in adrenal adenomas. Further studies in normal human adrenals are needed to confirm these findings and to investigate the role of miRNAs in aldosterone biosynthesis.

SINGLE NUCLEOTIDE POLYMORPHISM, EPISTATIC AND SEX-DEPENDENT ASSOCIATION ANALYSES OF GENES OF THE RENIN-ANGIOTENSIN SYSTEM AND BLOOD PRESSURE IN FAMILIES
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Background: Genes encoding key elements of the renin-angiotensin system (RAS) cascade have been previously, but inconsistently, associated with blood pressure. Comprehensive gene-wide association analyses of RAS genes have not typically been performed, nor have associations been confirmed.

Aim: To determine whether individual single nucleotide polymorphisms (SNPs) in RAS genes are associated with blood pressure, and that sex interactions between these RAS genes are not similarly associated with blood pressure, but that sex interactions may be important and should be investigated further.

Methods: 90 SNPs from the 5 RAS genes were genotyped using leucocyte DNA from 2,969 blood pressure (SBP).

Results: Mean age was 65 years (SD 13). SBP and DBP were significantly associated with SNPs in CYP11B2 (β=0.01–0.05). Initial results also indicated that these SNPs show strong evidence of differential association with SBP for males and females (sex-SNP interaction, P=0.005–0.015).

Conclusion: Our analyses confirm that several SNPs in and around key RAS genes are associated with SBP. They suggest that epistatic interactions between these RAS genes are not similarly associated with blood pressure, but that sex interactions may be important and should be investigated further.

EFFECT OF AGING ON ASCENDING AORTIC FLOW MEASURED BY NON-INVASIVE MRI
Adji A*, Kachenoura N*, Bollache E*, Avolio AP, O’Rourke MP*, Mousseaux E*

Background: Limited invasive (electromagnetic) ascending aortic (AA) flow wave contour indicates impaired left ventricular (LV) function in the elderly. However, conventional non-invasive (Doppler) recordings have not shown similar aging change.

Aim: To illustrate changes in AA flow and carotid pressure waves in an apparently normal cohort, using cardiac magnetic resonance imaging (CMRI).

Methods: Fifty subjects (aged 21–70 years; 28 males) underwent velocity-encoded CMRI of the thoracic aorta using a 1.5T system (Sigma, GEMS, Waukesha, USA). AA flow was measured non-invasively by subtracting simultaneous forward and backward flow velocity across the AA cross-section. DR½ was determined as ratio of difference in flow velocity between systolic peak and one-third of deceleration phase to peak flow velocity. Comparisons were made between young (<50 years; n=30) and older subjects (>50 years; n=20).

Results: Peak AA flow velocity decreased substantially (P<0.0001) from young (66.7 ± 17.7 cm/s) to older subjects (47.7 ± 12.7 cm/s). This could be associated with the age-related increase in aortic diameter. The substantial differences between CMRI flow waveforms and Doppler are due to the use of the outer flow envelope (highest particle flow) to estimate flow across the aortic cross-section with Doppler. However, late systolic flow was relatively lower in the older than in the younger subject group, with flow concavely in older persons (corresponding to greater augmentation of the pressure waveform), represented as DR½, which declined from 78% (c50 years) to 69% (>50 years) (P<0.001). These features are explicable on the basis of LV impairment from early and increased wave reflection during systole, and suggest reduction of wave reflection as a strategy for treating heart failure in the elderly.

Conclusion: AA flow waves recorded by CMRI showed aging changes, which are not apparent in conventional Doppler flow patterns. Our finding warrants further use of AA CMRI flow and pressure waveforms to characterize aging changes and ill-effects of stiffened central arteries. Findings support predictions of Westerhof and O’Rourke (J Hypertens 1996;13:943–952) and the force/velocity interpretations of Miyashita et al (Heart Vessels 1994;9:30–39) and Chiu et al. (Circ Res 1992;70:530–535).

NON-INVASIVE DETERMINATION OF ASCENDING AORTIC IMPEDANCE
Kachenoura N*, Bollache E*, Adji A*, Avolio AP, O’Rourke MP*, Mousseaux E*

Background: Non-invasive studies of ascending aortic (AA) impedance, using Doppler flow and carotid tonometry have not confirmed the aging changes demonstrated with invasive electromagnetic flow/micromanometer catheters in humans.

Aim: To determine AA impedance from cardiac magnetic resonance imaging (CMRI) flow recorded non-invasively and carotid pressure waveforms, and compare results with previously reported invasive data and realistic arterial model (O’Rourke and Avolio, Circ Res 1980;46;363–372).

Methods: Fifty apparently normal subjects (aged 21–70 years; 28 males) underwent velocity-encoded CMRI of the thoracic aorta using a 1.5T system (Sigma, GEMS, Waukesha, USA). AA flow was measured non-invasively by subtracting simultaneous forward and backward flow velocity across the AA cross-section. Impedance was determined by relating modulus and phase, corresponding frequency components of the AA flow waveforms with tonometric carotid pressure waveforms (used as surrogate of AA pressure) and recorded electronically, with the force/volume interpretation of Miyashita et al (Heart Vessels 1994;9:30–39) and Chiu et al (Circ Res 1992;70:530–535).

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Recumbent SST was positive (plasma aldo >140 pmol/l at 4 PA (FST negative) tested negative by seated and recumbent SST.

Results:

- After assuming recumbency) and (2) seated, with SSTs spaced at least 2 weeks apart, and

Conclusion:

Seated SST appears to be superior to recumbent SST in detecting PA and may be a reliable alternative to FST.

H-015

HYPERTENSION APLAQUE INSTABILITY IN A NOVEL HYPERTENSIVE ATHEROSCLEROTIC MOUSE MODEL

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Background: Hypertension is a significant risk factor for heart disease and the single biggest contributing factor for ischemic stroke. We have shown previously that an acute increase in pressure induces leukocyte adhesion and inflammation, but whether chronic high blood pressure affects plaque pathology is unknown.

Aim: In the current study, we explored the chronic effect of high blood pressure on plaque progression and examined whether blocking inflammation with the P-selectin blocking antibody RB40.34 can reduce plaque development in a novel spontaneously hypertensive, diet-induced atherosclerotic mouse model.

Methods: Spontaneously hypertensive BPH/2J mice were crossed with Apoe<sup>–/–</sup> mice to develop a fixed line of hypertensive mice with a deficiency of the Apo gene (BPH/Apo<sup>–/–</sup>). 8-week-old BPH/Apo<sup>–/–</sup> or Apo<sup>–/–</sup> mice were fed a high fat rodent diet (HFD) 21% and 0.15% cholesterol for 12 weeks.

Results: 24-hour averages of arterial pressure (AP), systolic AP (SAP), diastolic AP (DAP) and mean AP (MAP), determined with telemetry, were higher in BPH/Apo<sup>–/–</sup> compared to Apo<sup>–/–</sup> mice (13–15 mmHg; n=5; P<0.05). Total plaque area was similar in BPH/Apo<sup>–/–</sup> and Apo<sup>–/–</sup> mice (n=4–6; P>0.05). However, aortic sinus lesions from BPH/Apo<sup>–/–</sup> mice had greater lipid deposition (Oil red O; 18.1 ±2.4% vs 29.3 ±3.4%; n=4–6; P<0.05) and macrophage content (CD68; 13.9 ±2.0% vs 31.4 ±4.6%; n=3–5; P<0.05) compared to Apo<sup>–/–</sup> mice. Collagen content trended towards a reduction in BPH/Apo<sup>–/–</sup> compared to Apo<sup>–/–</sup> mice (11.7 ±2.4% vs 6.1 ±1.2%; n=3–4; P<0.09).

Collectively, these results suggest BPH/Apo<sup>–/–</sup> mice have reduced plaque stability. Treatment with two injections of RB40.34 (100 µg, i.p.) at week 1 and 3 did not affect total or regional plaque area, lipid deposition or macrophage content (n=5–11; P>0.05).

However, whilst there was no change in collagen content in Apo<sup>–/–</sup> mice (n=3–6; P>0.05), BPH/Apo<sup>–/–</sup> mice demonstrated increased collagen with RB40.34 treatment (control: 5.1 ±1.0% vs RB40.34: 20.5 ±4.2%; n=4–5; P<0.01). Plaque stability, as measured by the collagen/lipid ratio, was also improved with RB40.34 treatment in the BPH/Apo<sup>–/–</sup> mice (control: 0.20 ±0.06 vs RB40.34: 0.93 ±0.20; n=4–5; P<0.01) but not in Apo<sup>–/–</sup> mice.

Conclusion: Chronic high blood pressure reduces plaque stability, which in the presence of hypertension, is partially improved by anti-inflammatory therapy. Our data suggest that targeting leukocyte recruitment may be beneficial for the long-term management of heart disease in hypertensive patients.

H-016

ADENOSINE AND LIDOCaine COMBINATION I MPROVES AORTIC RING PRESERVATION AFTER 6-DAY COLD STORAge

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Background: Vessel allograft is often stored in cold medium for few hours to several days for revascularization or replacement of infected vessels. Maintenance of vascular quality and function, however, following extended storage for 24–48 hours remains unsatisfactory.

Adenosine-lidocaine (AL) with melatonin (M) and insulin (I) in low Ca<sup>2+</sup>/high Mg<sup>2+</sup> Krebs-Henselet (modified HK) solution has been shown to improve heart function after prolonged preservation.

Aim: To examine the effect of AL with or without MI combination in modified KH on rat aorta physiological functions after 6 days of cold storage.

Methods: Thoracic rat aortic rings (3 mm) stored in cold (4°C) KH, modified KH, modified KH+AL, and modified KH+ALMI solutions for 6 days. At the end of cold storage, all segments were transferred to KH-filled organ bath and rewarmed to 37°C. The contractility and relaxation functions of preserved rings were measured and compared with freshly harvested rings (controls) using isometric force transducer. Values were expressed as the percentage of tension/relaxation of controls.

Results: After 6 days of cold storage, aortic rings preserved in modified KH lose ~55% and ~65% of their contractile response to NE and KC, respectively; while those in modified KH only experienced ~10% and 25% loss. AL or ALMI in modified KH fully restored the contractility response of the rings to NE (100%), but were unaltered with KC (~80%). Relaxations to acetylcholine (Ach) were 42%, 80%, 93% and 70% for KH, modified KH, modified KH+AL and modified KH+ALMI, respectively. All rings except those in KH could achieve 100% relaxation with nitric oxide.

Conclusion: After 6-day cold storage, low Ca<sup>2+</sup>/Mg<sup>2+</sup> ratio of KH (modified KH) somewhat improved vascular reactivity. Adding AL and ALMI seemed to further increase vascular contractility, especially in response to NE. Yet, only AL treatment significantly improved Ach-induced relaxation compared to standard KH, which may indicate improved endothelial preservation.
H-017
POTENT VASODILATORY EFFECT OF ADENOSINE-LIDOCAINE (AL) INDEPENDENT OF INTRACORONARY ENDOTHELIUM: POSSIBLE ROLE OF Kv AND mitoKATP CHANNELS
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Background: Vasodilator therapy has been used in attempt to prevent or to dilate graft spasm. Some vasodilators, however, require functional endothelium and become ineffective if the endothelial layer is damaged during graft harvesting. Adenosine and lidocaine are both vasoactive agents and the combination (AL) has been shown to induce coronary dilatation.

Aims: (1) To examine the vasodilatory effect of AL solution compared with adenosine or lidocaine in endothelium intact and denuded rat aortic rings. (2) To investigate the possible involvement of potassium channels in AL vasodilatory action.

Methods: Endothelium intact or denuded thoracic rat aortic rings (3mm) was equilibrated in a Krebs Henseleit-filled organ bath, bubbled with 95% O2 and 5% CO2 at 37°C. After precontracted with norepinephrine (NE), concentration relaxation curves to adenosine, lidocaine or AL (100–1000 µM) were recorded using an isometric force transducer. Another series of aortic rings was examined to observe AL relaxation with and without specific Kv and mitoKATP channel blockers. Relaxant responses were expressed as percentage of maximal relaxation to 1000 µM papaverine.

Results: In intact rings, lidocaine produced 65.6% dilation at maximal concentration of 1000 µM compared to 100% and 97.8% relaxation with adenosine and AL, respectively. Adenosine relaxation, however, was attenuated after endothelial removal (89.6%), whereas lidocaine-induced relaxation remained unchanged (65.4%). Interestingly, maximal (100%) relaxation was achieved with ADP regardless of the absence of endothelium. The relaxation effect of AL was significantly inhibited by Kv and mitoKATP channel blockers in endothelium-denuded aortic ring, but not when endothelium was intact.

Conclusion: AL and adenosine are potent vasodilators in rat aortic rings, while lidocaine is less potent. Unlike adenosine, AL relaxation was endothelium independent. Endothelium-independent relaxation of AL may involve Kv and mitoKATP channel activation.

H-018
CENTRAL AORTIC PRESSURE AND ASSESSMENT OF BARORECEPTOR FUNCTION
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Background: Non-invasive assessment of baroreflex sensitivity (BRS) is conventionally performed by relating changes in heart rate (HR) and systolic pressure (SBP), where continuous beat-to-beat changes in blood pressure are measured in a peripheral location (e.g., finger). The pressure pulse is, however, amplified between the aorta and periphery, with amplification depending on HR due to the frequency dependency of the brachial transfer function. In addition, the degree of pulse amplification reduces with increased aortic stiffness as occurs with age.

Aim: To assess BRS using changes in SBP and pulse interval (PI) and to compare values of BRS computed from peripheral (finger) SBP (pSBP) and corresponding central aortic SBP (cSBP).

Methods: Continuous blood pressure and ECG signal were acquired in 6 adult subjects (mean age 42±16 years) over periods of 5–20 seconds. The spontaneous sequence technique was used for computation of BRS from the slopes of linear relationships of SBP and PI of contigous cardiac cycles, where SBP and PI change in the same direction. Central aortic pressure was determined from the finger pressure pulse (Fimometer) by applying a mathematical transfer function. BRS was computed for a lag of 0, 1, 2 and 3 cardiac cycles between PI and SBP.

Results: The relationship between BRS computed from cSBP (y) and from pSBP (x) was y = 0.98x + 0.19 (r²=0.84). However, for all computed BRS values (n=24), BRS using cSBP were higher (by 21.5±2.95 SD%) in 62.5% of measurements (15/24) and lower (by 23.1±12.9 SD%) in 37.5% (9/24). Across subjects, there was a trend for the difference to be positively related to heart rate (r=0.21) and negatively related to age (r=0.24) and mean arterial pressure (r=0.11).

Conclusions: BRS computed from changes in HR and cSBP show a relation to values computed from pSBP. The difference between the two can, however, be positive or negative, and is related to hemodynamic parameters and age. Further studies are required to assess underlying mechanisms of the differences and potential clinical significance.

H-019
ETHANOL IMPAIRS EXPRESSION OF ESTRUS RECEPTORS AND INCREASES ICAM-1 AND GALECTIN-3 IN HUMAN UMBILICAL VEIN AND CEREBRAL MICROVASCULAR ENDOTHELIAL CELLS
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Background: Endothelial cells play a pivotal role in maintaining vascular homeostasis and avoiding dysmorphic remodeling. One underlying mechanism of cardiovascular disease is the expression of estrogen receptors (ERs) and inflammatory markers (ICAM-1, galectin-3). The expression of ERs, ICAM-1 and galectin-3 are two different types of endothelial cells: human umbilical vein endotheilial cells (HUVEC), as a model of large vessel endothelial cells, and cerebral microvascular endothelial cells (hMEC), which are associated with the blood-brain barrier.

Aim: To determine the effects of ethanol (EtOH) on the expression of estrogen receptors (ERs) on ERα and -ERβ1, galectin-3 and ICAM-1.

Methods: Cells were treated with EtOH and were then evaluated for the expression of ERα and -ERβ1, galectin-3 and ICAM-1 using Western blot assay.

Results: Exposure to EtOH increased the expression of ERα, but did not change ERβ1 (ERβ1 was not detected in the HUVEC line used in this study). In terms of inflammatory markers, EtOH increased ICAM-1 and galectin-3 in both cell lines and activated p-IκBα in hMEC.

Conclusion: These findings suggest that alcohol accelerates ED by decreasing estrogenic effects and increasing inflammatory response in both the systemic and cerebral vasculature.

H-020
THE PROTECTIVE EFFECT OF APOPLP-PROTEIN A-I ON MEAN ARTERIAL PRESSURE IN AN IN VIVO MODEL OF CYTOKINE-INDUCED HYPERTENSION
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Background: Failure of the trophoblast to appropriately invade uterine spiral arteries is an initiating event in preeclampsia (hypertension in pregnancy). The pro-inflammatory cytokine TNF-α inhibits trophoblast invasion. Previous work has demonstrated a protective effect of apolipoprotein A-I (apoA-I) against the deleterious effects of TNF-α on the uterine endometrial cell interactions in vitro. The present study investigated the protective effect of apoA-I on mean arterial pressure (MAP) via radiotherapy in vivo.

Aim: To determine if apoA-I can reverse the deleterious effects of TNF-α on MAP using the cytokine-induced model of hypertension in pregnancy in the mouse.

Methods: Mice were time-mated and randomly assigned to treatment groups (n=3 per group). Saline or lipid-free apoA-I was administered at a dose of 40 mg/kg via the tail vein on gestation days (gd) 10.5 and 12.5. A mini-osmotic pump that released either saline or TNF-α (500 ng/kg/day) was implanted on gd13.5. Mice were implanted with radio-telemetry sensors prior to pregnancy and their MAP recorded throughout gestation.

Results: The animals administered lipid-free apoA-I, prior to the TNF-α insult, showed a significantly smaller rise from baseline (0.99 ± 0.79SD mmHg) in their mean arterial pressure than the TNF-α treated animals (8.84 ± 0.65SD mmHg; P<0.05) upon infusion of TNF-α on gd13.5. On gd17, the rise in MAP from baseline of TNF-α treated animals (9.81 ± 3.05SD mmHg) was significantly higher than that of animals administered with lipid-free apoA-I (5.4 ± 0.72SD mmHg; P<0.05).

Conclusion: Although the lipid-free apoA-I did not fully normalize their MAP, these animals showed a significantly smaller rise in MAP than the TNF-α-only treated animals. Lipid-free apoA-I confers at least a partially protective effect against cytokine-induced hypertension in a whole animal in the presence of a fetus.

H-021
DOES STEM CELL COMBINATION THERAPY ENHANCE CORONARY VESSEL NUMBER IN A RODENT HEART FAILURE MODEL MORE THAN MONOTHERAPY?
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Background: Transplantation of various stem cells and angiogenic stimulation have both shown to improve heart function after myocardial infarction (MI). While stem cells are being used to regenerate cardiac muscle and contractile function, including skeletal myoblast sheets, most therapies do not promote revascularization. Omentum provides a rich source of endogenous angiogenic-inducer factors for revascularization.

Aim: To investigate if myoblast-omentum combination therapy improves the number and endothelial function of coronary arterial vessels within and adjacent to the infarct zone of rat hearts 3–6 weeks post-treatment in rats more than monotherapy with either treatment alone.

Methods: Lewis male rats (10 weeks old) were allocated to four treatment groups 2 weeks after inducing MI under anesthesia: control, no treatment (n=4), combined treatment (0-M, n=5), myoblast sheets (n=5) and omentum (n=6). All rats were treated with synchrotron coronary microangiography in vivo during baseline, acetycholine, sodium nitroprusside and dobutamine infusions a further 3–6 weeks later.
**H-022**

**BRACHIAL-TO-RADIAL SYSTOLIC BLOOD PRESSURE AMPLIFICATION IS SIGNIFICANTLY BLUNTED IN PATIENTS WITH TYPE 2 DIABETES: UPPER LIMB HEMODYNAMICS HAVE AN INFLUENTIAL ROLE**

Clime R., Picone DS, Keske M., Sharaman JE

**Methods:** We found recently significant age-related increases in brachial-to-radial systolic blood pressure amplitude (Bra-Rad-SBPamp). This has implications for correct central SBP estimation. Patients with type 2 diabetes (T2D) have vascular irregularities that may alter Bra-Rad-SBPamp.

**Aim:** To determine the magnitude and correlates of Bra-Rad-SBPamp in T2D.

**Methods:** Twenty T2D (64±8 years; 50% male) and 20 controls (60±8 years; 50% male) were recruited. Blood pressure waveforms were calibrated using simultaneous ultrasound imaging of the brachial and radial arteries. The 1st Korotkoff sound (denoting SBP) at each arterial site was identified from the first inflection point of Doppler flow during BP cuff deflation. Bra-Rad-SBPamp was calculated by radial minus brachial SBP. Local and systemic hemodynamics were recorded by tonometry and ultrasound.

**Results:** Bra-Rad-SBPamp was higher than brachial SBP for both T2D (213±8 vs 144±7 mmHg; P=0.023) and controls (211±10 vs 144±7 mmHg; P=0.042). Central SBP was significantly higher in both controls and T2D when radial pressure waveforms were calibrated using radial, compared with brachial SBP (both P<0.001). The product of brachial arterial flow velocity and diameter was significantly increased in T2D (213±10 vs 144±7 mmHg; P=0.023), and this was inversely correlated with Bra-Rad-SBPamp (r=−0.643; P=0.003) even after adjustment for age and sex (β=−0.031, adjusted R2=0.366; P=0.002).

**Conclusions:** Patients with T2D have higher radial SBP than brachial SBP, but compared with controls, overall Bra-Rad-SBPamp is significantly blunted. Local hemodynamics influence the magnitude of Bra-Rad-SBPamp and overall these findings have implications regarding correct estimation of central BP.

**H-024**

**CARDIAC STABILITY AND HEMODYNAMIC RECOVERY WITH ADENOSINE-LIDOCAINE/MG2+ (ALM) FOLLOWING ASPHYXIAL CARDIAC ARREST DURING HYPOTERMIA AND REWARMING**

Dedner VF, Dobson GP

**Background:** Cardiac arrest claims average 17 million lives per year globally and the survival rate remains low (~10%). While therapeutic hypothermia followed by rewarming continues to gain popularity in resuscitation protocols, the efficacy of the standard drug, epinephrine, has been questioned due to potential risk of post-arrest myocardial dysfunction. Adenosine-lidoacaine/Mg2+ (ALM) has been shown to provide cardio-protection and resuscitation following electrical-induced cardiac arrest and hemorrhagic shock.

**Aim:** To examine the effects of ALM compared to epinephrine on return of spontaneous circulation (ROSC) and post-ROSC hemodynamics following 8 min asphyxial cardiac arrest during hypothermia and rewarming.

**Methods:** Non-heparinized Sprague-Dawley rats (n=24) were randomly assigned to ALM and epinephrine (0.01 mg/kg BB) groups. Asphyxial cardiac arrest (MAP<10 mmHg) with hypothermia (33°C) was induced for 6 minutes followed by resuscitation (0.5 ml iv bolus and chest compressions) and rewarming. Arterial pressures (systolic, diastolic, and mean (MAP)) and heart rate (HR) during and post resuscitation were recorded.

**Results:** Despite lower arterial pressures during chest compressions, all rats in the ALM group were resuscitated, while 25% of epinephrine-treated rats did not achieve ROSC due to persistent ventilatory fibrillation (VF). None of ALM rats experienced VF during and post resuscitation. Immediately after ROSC, the epinephrine group had elevated MAP and HR, whereas those in the ALM group MAP and HR were moderately low (MAP 129±7.0 vs 59±5.5 mmHg; HR 151±13.6 vs 59±5.5 bpm). Hemodynamics in the ALM group gradually increased, leading to significantly higher MAP with similar HR after 120 min post ROSC.

**Conclusion:** Epinephrine increased arterial pressures, HR and VF incidence during resuscitation. In contrast, ALM lowered arterial pressures and HR to a certain level, leading to improved cardiac stability and hemodynamic recovery after 2 hours of ROSC compared to epinephrine.

**H-025**

**INVESTIGATION OF THE ROLE OF RENAL LONG NON-CODING RNA IN ESSENTIAL HYPERTENSION THROUGH THE USE OF RNA-SEQ**

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**Background:** The kidney has long been thought to be involved in the etiology of essential hypertension (EH). The cause may involve genetic variants that are classified as long non-coding (lnc) RNAs, which can act over long distances and across chromosomes to activate transcription of genes that can have consequences for various disease.

**Aim:** To perform genome-wide RNA sequencing of renal RNAs to determine which lncRNA are differentially expressed between kidneys of 15 untreated EH and 15 normotensive (NT) white European subjects.

**Methods:** Stranded 100bp pair end RNA libraries were sequenced on an Illumina HiSeq 2000 platform following polyA purification and ribosomal depletion. Bioinformatic analysis for differential expression involved demultiplexing, alignment, RNA counting and normalization. Reads were aligned against the human genome (build version HS19) using the tophat aligner (http://tophat.cbcb.umd.edu). Transcripts were assembled using reference-based annotation by Partek software and filtered from the UCSC genome browser. lncRNA were counted, normalized and analysed for the identification of differential expression.

**Results:** The expression of IncRNA was very low compared to other RNAs. We found 10 transcripts that were differentially expressed between normotensive and hypertensive subjects. Some of these were located in EH quantitative trait loci from the hypertension GWAS meta-analyses. These were LOC100130417, LIN000152, LOC339975, LOC100507584, LOC1041463, LIN000035, LOC100507217, LOC936933, LOC1041463, LOC938868 and LOC339849.

**Conclusion:** We identified 10 IncRNAs that were differentially expressed in EH kidneys. This is the first study to show that IncRNA may be involved in the etiology of EH in humans.
THE ROLE OF THE AT2 RECEPTOR IN MONOCYTE ACTIVATION AND MACROPHAGE POLARIZATION


Aim: To determine whether AT2R stimulation influences monocyte activation and macrophage polarization.

Methods: Whole human blood was pre-treated with the AT2R agonist Compound 21 (C21, 1 μM) prior to monocyte activation. Flow cytometry analysis (2D-FC/CD11b) showed that LPS-induced monocyte activation (23±1.5% vs control 100%; n=4; P<0.05) was unchanged following C21 pre-treatment. In separate experiments, human primary monocytes from buffy coats were differentiated into macrophages via M-CSF (100 ng/mL) for 7 days, and polarized using IFNγ (20 ng/mL)/LPS (100 ng/mL) to induce M1 macrophages, and IL-4 (20 ng/mL) to induce M2 macrophages, in the presence and absence of C21 (1 μM). Macrophage phenotype was determined via flow cytometry after 24 h and gene expression after 6 h.

Results: IFNγ/LPS increased CD64 expression 1.5±0.1-fold compared to unstimulated macrophages (M0; n=8; P<0.001), and IL-4 increased C2026 and C2020R expression 1.2±0.0- and 2.1±0.2-fold compared to M0 control, respectively (n=5; P<0.01). C21 pre-treatment, however, had no effect on either M1 or M2 cell surface marker expression. IFNγ/LPS increased M1 marker TNFα and IL-6 gene expression to 43.±8.0 and 23.5±6.0-fold compared to M0 control, respectively (n=5–8; P<0.01). C21 significantly reduced TNFα expression by 15.2±4.1-fold compared to M0 control (n=8; P<0.05) and had a tendency to reduce IL-6 expression (by 93.2±50.0-fold compared to M0 control; n=5; P=0.11). Gene expression of the M2 marker SRB1 was increased 8.1±2.2-fold compared to M0 control (n=5–6; P<0.05) in response to C21 pre-treatment and had no effect.

Conclusion: Direct AT2R activation does not influence monocyte activation nor macrophage surface marker phenotype, but may reduce M1 gene expression, warranting further exploration of the role of AT2R stimulation on macrophage function.

EFFECT OF AN AORTIC ELASTIC WRAP ON SYSTEMIC IMPEDANCE

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Aim: To investigate the effects of the AW on input impedance (Zin) and characteristic impedance (Zc) in a bench model of the arterial circulation.

Methods: A hydraulic loop model of the circulation was used. This is composed by a silicon model of the arterial tree connected to a pulsatile pump, which mimics physiological conditions (3 heart rates (HRs) of 50, 65 and 80 bpm; and 3 stroke volumes (SVs) of 60, 70 and 80 mL). The AA segment of the model was wrapped with a distensible elastic band (10 cm long), achieving a 30% diameter reduction of the AA at 0 mmHg. Flow waves were recorded at the inlet via a transonic flowmeter, and pressure waves were recorded at 10 locations along the model with a Millar microtip pressure catheter. Where (i=frequency) and Zc (initial slope of the P-Q curve) were calculated for all the possible combinations of HR and SV.

Results: After wrapping, there was a consistent reduction of Zc, with the exception of the 50 bpm HR–80 mL SV configuration. The maximum reduction (−4.1% compared with the corresponding unwrapped value) was achieved at the lowest HR (50 bpm) and lowest SV (50 mL). Zin modulus was reduced consistently by the AW, the 1st harmonic component showing a decrease (varying from −14.5% to −30.1% of the corresponding unwrapped value) for all the low (50 bpm) and medium (65 bpm) HR configurations.

Conclusion: Our in vitro modelling study shows that the AW procedure consistently decreases Zin and Zc for most HR and SV configurations, resulting in a concomitant decrease of PP. This suggests a potential use of the AW as a non-pharmacological treatment of isolated systolic hypertension. The dependency of the impedance reduction on HR and SV indicates a potential patient-specific optimization approach.

MEASUREMENT OF RETINAL ARTERY PULSE WAVE VELOCITY IN THE RAT USING HIGH SPEED IMAGING

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Aim: To determine whether AT2R stimulation influences monocyte activation and macrophage polarization.

Background: Monocyte activation and macrophage infiltration into the vessel wall are pivotal to the development of atherosclerosis. Macrophages polarize into either M1 (classically-activated) or M2 (alternatively-activated) phenotypes, with higher levels of M1 macrophages present in unstable regions of human plaque. The angiotensin type 2 receptor (AT2R) can exert anti-inflammatory effects in atherosclerosis.

Methods: Whole human blood was pre-treated with the AT2R agonist Compound 21 (C21, 1 μM) prior to monocyte activation. Flow cytometry analysis (2D-FC/CD11b) showed that LPS-induced monocyte activation (23±1.5% vs control 100%; n=4; P<0.05) was unchanged following C21 pre-treatment. In separate experiments, human primary monocytes from buffy coats were differentiated into macrophages via M-CSF (100 ng/mL) for 7 days, and polarized using IFNγ (20 ng/mL)/LPS (100 ng/mL) to induce M1 macrophages, and IL-4 (20 ng/mL) to induce M2 macrophages, in the presence and absence of C21 (1 μM). Macrophage phenotype was determined via flow cytometry after 24 h and gene expression after 6 h.

Results: IFNγ/LPS increased CD64 expression 1.5±0.1-fold compared to unstimulated macrophages (M0; n=8; P<0.001), and IL-4 increased C2026 and C2020R expression 1.2±0.0- and 2.1±0.2-fold compared to M0 control, respectively (n=5; P<0.01). C21 pre-treatment, however, had no effect on either M1 or M2 cell surface marker expression. IFNγ/LPS increased M1 marker TNFα and IL-6 gene expression to 43.±8.0 and 23.5±6.0-fold compared to M0 control, respectively (n=5–8; P<0.01). C21 significantly reduced TNFα expression by 15.2±4.1-fold compared to M0 control (n=8; P<0.05) and had a tendency to reduce IL-6 expression (by 93.2±50.0-fold compared to M0 control; n=5; P=0.11). Gene expression of the M2 marker SRB1 was increased 8.1±2.2-fold compared to M0 control (n=5–6; P<0.05) in response to C21 pre-treatment and had no effect.

Conclusion: Direct AT2R activation does not influence monocyte activation nor macrophage surface marker phenotype, but may reduce M1 gene expression, warranting further exploration of the role of AT2R stimulation on macrophage function.

HYPERTENSION, WHITE COAT HYPTERTENSION AND MASKED HYPTERTENSION: AMBULATORY BLOOD PRESSURE FINDINGS FROM THE AUSSDIAB COHORT (WAVE III)

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Background: The Australian Diabetes, Obesity and Lifestyle Study (AusDiab) is an Australian national, population-based study of over 11,000 adults from all states and the Northern Territory of Australia that began in 1999–2000 and was designed to assess the risks of all-cause and CVD mortality. The third wave of assessment carried out in 2011–2012 provided the opportunity to compare office and out-of-office blood pressure (BP) using ambulatory BP monitoring (ABPM) in an opportunistic sub-group drawn from the well described Australian cohort.

Aim: To compare clinic BP and ABPM in the AusDiab cohort at wave III.

Methods: During the wave III on-site assessment of 4614 AusDiab participants, 836 were approached and 586 agreed to undergo 24 hour ambulatory monitoring. Of these assessments, 491 were conducted successfully (>15 readings, >15 hours recording).

Results: Mean age was 59.7 years, mean BMI 27.5 kg/m², and 53% were women. The mean clinic systolic over diastolic BP was 127/73 mmHg and mean 24 hour BP was 89/69 mmHg, and average retinal artery diameter of 54.6±11 μm. A positive correlation was obtained between arterial diameter and pulse amplitude (r²=0.52). The correlation between rPWV and HR was r²=0.32.

Conclusion: This study confirmed the feasibility of a novel approach to measure rPWV in animal models. This technique is a promising accessible tool for microvascular assessment of PWV. The inter-individual variability is likely related to variations in depth of anesthesia and blood pressure which needs to be controlled to improve reproducibility. Future studies are required to assess the significance of PWV in the retinal microvasculature in rat models of cardiovascular disease.

THE ROLE OF T CELLS AND MACROPHAGES IN PERIVASCULAR SYMPATHETIC NERVE GROWTH AND THE DEVELOPMENT OF NEUROGENIC HYPERTENSION IN OBESITY

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Introduction: Hypertension in obesity occurs as a result of abnormal increases in sympathetic nerve density over arteries controlling blood pressure; the causative factors underlying these changes are unknown.

Aim: To examine whether T cells drive obesity-induced sympathetic hyperinnervation and the development of neurogenic hypertension in obesity.

Methods: C57BL/6 and Rag1−/− mice, which lack mature B and T cells, were fed normal chow or a high fat diet (HFD). Mesenteric arteries were isolated and pressure myography, electrophysiology and videomicroscopy was used to measure intraluminal pressure, membrane brain potential and vessel diameter. Nerves were activated by electrical field stimulation. Immunohistochemistry was performed using antibodies against nerve growth factor (NGF), COX-3 (T cells) and F4/80 (macrophages). Sympathetic innervation was examined over both mesenteric and renal arteries using synaptophysin (synaptic vesicles). Images were collected using confocal microscopy and analysed using Image J.

Results: Obese C57BL/6 mice were hypertensive. Nerve-mediated contractions and excitatory junction potentials (EJP) were augmented and the perivascular sympathetic nerve plexus became denser. Numbers of perivascular NGF-producing immune cells (T cells and macrophages) were increased, infiltration of these cells was shown to precede expansion of the nerve plexus. Rag1−/− mice fed a HFD also became obese. Numbers of perivascular NGF-producing immune cells were not, however, increased, arteries were not hyperinnervated and mice remained normotensive.

Conclusion: Nerve growth factor producing T cells and macrophages drive sympathetic hyperinnervation and neurogenic hypertension in obese C57BL/6 mice.
120/72 mmHg; 39% were hypertensive based on 14% having ABPM mean 24h >130/80 mmHg; 42% were on antihypertensive therapy (25%). Both office and ambulatory BP were reducible in higher in men (office 4.5/6.5, 24h ±6.4/5.2 mmHg). These differences persisted after adjustment for age. The prevalence of white-coat hypertension was 12% and masked hypertension was 4%. Based on ABPM, 14% had untreated hypertension and 11% of those treated were still hypertensive, which was 3 times more common in men than in women (19% vs 6%).

Conclusions: 24 hour ABPM findings in this sub-group of the AusDiab cohort at wave III showed that one in three have blood pressure above target, despite a high prevalence of antihypertensive treatment. One in 8 have white coat hypertension, but the level of masked hypertension appears to be relatively low. These findings highlight the importance of out-of-office BP assessments in hypertension management.

**VASCULAR FUNCTION IN THE STROKE-PRONE SPONTANEOUSLY HYPERTENSIVE RAT IS Y CHROMOSOME DEPENDENT**

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Background: The origin of the Y chromosome accounts for 15–20 mmHg difference in arterial pressure. The gene(s) and mechanism(s) underlying this effect, however, remain unknown. As vascular dysfunction is a hallmark of hypertension, we hypothesize that vascular dysfunction in the stroke-prone spontaneously hypertensive rat (SHRSP) may be influenced by the Y chromosome.

Aim: Using the Y cosmic approach, which exclusively replaces the Y chromosome of the SHRSP with the normotensive WKY Y chromosome (SP/WKY-Y) and vice versa (WKY/SP-Y), we aimed to examine the influence of the origin of the Y chromosome on vascular function.

Methods: Thoracic aortas were excised from rats and mounted in organ baths.

Results: Concentration-vasoactivity curves to acetylcholine (ACH), and sodium nitroprusside (SNP) revealed impaired endothelium-dependent (ACH), but not endothelium-independent (SNP), vasorelaxation in the SHRSP aorta compared to the WKY aorta (EC50: SHRSP 17.9±8.2 mmHg, WKY 7.6±1.7 mmHg, P<0.01; 100μM SNP: SHRSP 6.9±1.8 mmHg, WKY 7.3±0.8 mmHg, P<0.01). Replacement of the SHRSP Y chromosome with the WKY Y chromosome (SP/WKY-Y) significantly improved ACH-induced vasorelaxation (EC50 of SP/WKY-Y 2.7±0.1; n=6; P<0.001). Importantly, prostaglandin blockage with indomethacin (10 μM) abolished differences in endothelium-dependent vasodilation, implicating a role for the origin of the Y chromosome in determining constriction prostanoid release. Furthermore, contractions to L-NAME were impaired in the SHRSP (16.4±16.2SD % of maximum; n=5) compared to WKY (70.5±19.2SD % of maximum; n=3) and SP/WKY-Y aortas (57.8±11SD % of maximum; n=8; P=0.03), suggesting the origin of the Y chromosome also modulates basal NO levels. Responses to the vasoconstrictor angiotensin II (100μM) were similar, despite differences in circulating angiotensin II levels (WKY 44.3±4.75D %, SHRSP 30.2±8.2SD %, SP/WKY-Y 58.3±7.9SD %, contraction from baseline: n=5–9; P>0.5).

Conclusion: We provide evidence that the origin of the Y chromosome influences endothelium-dependent vasodilation by regulating constrictor prostanoid release and basal NO levels.

**AMBULATORY AND CENTRAL HEMODYNAMICS ARE ELEVATED DURING PROGRESSION TREKKING ASCENT TO HIGH-ALTITUDE AND ASSOCIATED HYPOXIA**

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Background: High-altitude hypoxia may cause temporary increases in resting brachial blood pressure (BP) that reflect on more sensitive measures of BP control (24 hour ambulatory BP and resting central BP) is largely unknown.

Aim: To determine 24 hour ambulatory BP and resting central BP, as well as haemodynamic correlates of acute mountain sickness (AMS) during a progressive ascent to high-altitude.

Methods: Measures of oxygen saturation (pulse oximetry), 24 hour ambulatory BP (AOD), resting brachial and central BP (Pulseco) were recorded in 10 adults aged 27 ± 4 years (30% male) during a 8-day trek to Ms. Everest base camp, Nepal. Data were recorded at sea level (stage 1: ~450 m above sea level (ASL)) and at progressive ascension to 3440 m ASL (stage 2), 4350 m ASL (stage 3) and 5164 m ASL (stage 4). The Lake Louise Score (LLS) was used to quantify AMS symptoms.

Results: Total LLS increased step-wise from sea level to stage 4 (0.3±0.7 vs 4.4±2.0; P<0.012), whilst oxygen saturation decreased to 77 ± 9% (P<0.001). The highest recordings of 24 hour ambulatory, daytime, night-time, brachial and central systolic BP and diastolic BP were achieved at stage 3. These were significantly greater than at sea level (P<0.005 for all), 24 hour ambulatory heart rate and night heart rate correlated with oxygen saturation (r=0.741 and -0.608, both P<0.001) and total LLS (r=-0.648 and r=-0.493; both P<0.001).

Conclusion: 24 hour ambulatory BP, central BP and heart rate are elevated during high-altitude hypoxia, but AMS symptoms appear only to tachycardia.

**DEPLETION OF B CELLS BY AN ANTI-C020 MONOCLONAL ANTIBODY INHIBITS ANGIOTENSIN II-INDUCED HYPERTENSION IN MICE**

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Background: Lympoche-deicient Rag1−/− mice are protected from angiotensin II (Ang II)-induced hypertension. Adoptive transfer studies suggest that this protective effect may be due to the lack of T cells, and not to a lack of B cells. Two major subsets of B cells exist. B2 cells produce antigen-specific antibodies and thus represent an important component of the adaptive immune response. By contrast, B1 cells generate non-specific (natural) antibodies, thus forming part of the innate immune response.

Aims: To re-assess the role of B cells in Ang II-induced hypertension in mice by their depletion with anti-C020 monoclonal antibody.

Methods: Mice were infused with Ang II (0.7 mg/kg/day) for 28 days and B1, B2 and T cells were measured in aorta, kidney and spleen by flow cytometry.

Results: Subcutaneous infusion of Ang II (0.7 mg/kg/day) for 28 days resulted in a markedly eddy systolic BP than that in saline-treated controls (175 ± 5 mmHg versus 119 ± 3 mmHg, respectively, P<0.05; n=9). Flow cytometric analysis revealed the presence of B2 cells (CD19+CD22+) in the aorta, kidney and spleen, and B1 cells (CD19+CD11b−CD5+) in the peritoneal cavity of Ang II-treated mice. The numbers of B2 cells in these locations were not, however, different from the number seen in the saline-treated controls. Treatment of mice with an anti-mouse CD20 antibody (5 mg/kg, i.v.) one day prior to commencement of Ang II infusion and then 14 days later, reduced both B1 and B2 cell numbers by ~95% in the aorta, kidney, spleen and peripheral cavity (P<0.05; n=9) without affecting T cell (CD3+) levels. Importantly, B2 cell depletion blunted Ang II-induced increases in systolic BP by ~40% (i.e., 156 ± 8 mmHg in the Ang II–anti-mouse CD20 antibody group versus 177 ± 5 mmHg in the Ang II-control antibody group; P=0.05; n=9).

Conclusion: B cells are crucial for the development of Ang II-induced hypertension in mice. Future studies to elucidate the specific subset of B cells and immune mechanisms involved (e.g., antigen presentation, antibody and/or cytokine production) may uncover targets for novel anti-hypertensive therapies.

**THE EFFECTS OF POSITIVE ALLOSTERIC MODULATION OF GABAa RECEPTORS UPON STRESS AND HYPERTENSION IN SCHLAGER HYPERTENSIVE MICE**

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Background: An exaggerated pressor response to stress has been shown to be a predictor of the subsequent development of hypertension. Hypertensive Schlagler mice (BP/2Cr strain) will have neurogenic hypertension associated with abnormal reactivity of neurons in the forebrain integrating the response to adverse stress. Recent studies suggest they also have functional
and molecular differences in GABA<sub>α</sub> receptors compared with their normotensive counterparts (BPN/3J strain). Allopregnanolone is an endogenous neurosteroid reduced by chronic stress and, when administered, decreases anxiety by positive allosteric modulation of GABA<sub>α</sub> receptors.

**Aim:** To determine if allopregnanolone reduces the pressor effects of stress and basal MAP in BPN/2J mice.

**Methods:** Male BPN/2J (n=7) and BPN/2J (n=5) mice received vehicle or allopregnanolone (5mg/kg/day) via subcutaneous minipumps for a period of two weeks. Prior implantation of telemetry probes enabled recording of mean arterial pressure (MAP), heart rate (HR) and activity before and 7 and 14 days after minipump implantation. The cardiovascular response to awareness (dirty cage switch and restraint) and non-awareness (feeding) stress tests, as well as ganglion blockade with 5mg/kg pentolinium, were recorded before and 7–14 days after minipump implantation. Mice were perfused following stress and brains were removed for immunohistochemistry.

**Results:** In BPN/2J mice, 2 weeks of allopregnanolone reduced systolic arterial pressure (~6.8 mmHg; P=0.01) and attenuated the depressor response to pentolinium, whereas no effect on MAP or HR was observed in BPN/3J mice. Allopregnanolone produced marked reductions in the pressor response to both cage switch and feeding stress (~20%; P<0.01) in BPN/2J mice, whilst increasing the pressor response to awareness stress in BPN/3J mice (~33–48%; P<0.001). Stress-induced Fox decreases in the ventral medial amygdala and paraventricular nucleus were higher in untreated BPN/2J compared to BPN/3J mice. Allopregnanolone reduced Fox expression and abolished the difference between strains.

**Conclusions:** The selective antihypertensive and stress inhibitory effects of allopregnanolone in BPN/2J hypertensive mice suggests that allosteric modulation of GABA<sub>α</sub> receptors at the level of the hypothalamus and amygdala may be a major cause of hypotension in this model and may offer a possible new area for the development of therapy.

**Table 1:** Regression coefficients (β) for each JSBP, 95% confidence intervals and p values illustrating the effect of SBP and HTT at 4 time points on LVMI. Models adjusted for age, sex, clinic visit, HTT at that age, current BMI and presence of T2DM.

<table>
<thead>
<tr>
<th></th>
<th>β Coefficient</th>
<th>95% CI</th>
<th>p value</th>
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</thead>
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<tr>
<td>Current SBP</td>
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<tr>
<td>SBP at age 35</td>
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<td>0.05–0.12</td>
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<td>SBP at age 40</td>
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<td>SBP at age 45</td>
<td>0.04</td>
<td>0.00–0.08</td>
<td>&lt;0.05</td>
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**H-036**

**EFFECTS OF HEART RATE ON ARTERIAL STIFFNESS, CENTRAL HAEMODYNAMICS, CARDIAC OUTPUT AND TOTAL PERIPHERAL RESISTANCE**

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**Background:** Increased arterial stiffness is a marker for cardiovascular disease. Whilst numerous cross-sectional studies have found a pressure-independent effect of heart rate (HR) on arterial stiffness, others have found increased arterial stiffness with increased HR in the presence of mean arterial pressure (MAP) changes. The effect of HR on arterial stiffness has not, however, been studied previously in conjunction with cardiac output (CO) and total peripheral resistance (TPR) measurements.

**Aim:** To investigate the effects of acute, sympathetic-independent changes in HR on central pressures, CO and TPR.

**Methods:** 28 subjects aged 81±6.5 years (4 females) with anit cardiac pacemakers or implanted cardioverter defibrillators were studied. Each subject was paced in a random order from 60 to 100 beats per min (bpm), with 10 bpm increments. Brachial cuff-based pulse wave analysis was used to measure central (c) systolic (SBP), diastolic (DBP) and MAP and pulse wave velocity (PWV) was measured with a thoracic cuff and carotid tonometry (Sphygmocor® XCEL). TPR and stroke volume (SV) were derived from measured finger pressures, CO and TPR measurements.

**Results:** All parameters measured, except cSBP, changed significantly with HR (Table; mean±SEM). Despite a decrease in both cSV and CO, MAP increased in decreases in HR, PWV also increased with HR, but the significance was lost once corrected for changes in MAP (Table).

**Conclusion:** Acute, sympathetic-independent changes in HR raises CO and cDBP, which in turn increases MAP despite decreases in SV and TPR, and results in increased arterial stiffness.

**H-037**

**IS IT TIME FOR A NEW LOOK AT THE “WATCH AND WAIT” APPROACH — MID-LIFE ANTIHYPERTENSSIVE TREATMENT MAY NOT NORMALIZE LEFT VENTRICULAR MASS IN SPITE OF CONTROLLED BLOOD PRESSURE**

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**Background:** In cross-sectional studies, elevated blood pressure (BP) is associated with increased left ventricular mass (LVM), which leads to increased cardiovascular morbidity and mortality. Current practice involves a “watch and wait” approach in younger individuals with borderline-high BP. We investigated if this was a correct assumption.

**Methods:** 1,653 participants in the 1946 birth cohort underwent echocardiography at age 60–64 years and LVM, indexed to body-surface-area (LVM), was measured. The relationship between repeated measures of SBP and antihypertensive treatment (HTT—measured at 4 time points: 60–64 (current), 53, 43 and 36 years) and LVM at 60–64 years was examined using adjusted multiple regression models. Multilevel models of SBP were then used to estimate person-specific intercepts (SBP at 36 years) and slopes (rate of change in SBP between 36–60/64 years). The intercepts and slopes were included in sex-adjusted linear regression models with LVM as the outcome.

**Results:** Individuals on HTT, from 43 years onwards, had higher mean LVM than those who were not on treatment, irrespective of level of BP at the same age (Table). The effect of mid-life rate of change in BP (50–53 years) on LVM at age 60–64 years was 10 times greater than the effect of more recent rate of change (53 years–current age).

**Conclusions:** HTT does not normalize LVM in older individuals. This may be due to irreversible cardiac damage occurring in mid-life in poorly-controlled hypertensives. Early identification and treatment of individuals with rapidly increasing BP in mid-life may be key to preventing such damage. A review of current guidelines on monitoring and screening of BP may be required.
risks of death or dependency. This highlights the need for both the very rapid and sustained control of SBP in the first 7 days following ICH.

H-039

ASSOCIATION BETWEEN MAGNITUDE OF BLOOD PRESSURE REDUCTION AND CLINICAL OUTCOMES AFTER ACUTE INTRACRANIAL HEMORRHAGE: THE INTERACT2 TRIAL


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Background: The main phase of the INTERSTROKE blood pressure Reduction in Acute Cerebral haemorrhage Trial (INTERACT2) indicated that early intensive lowering of blood pressure (BP) was safe and improved functional outcomes in patients with acute intracerebral haemorrhage (ICH).

Aim: To examine whether larger BP reductions improve clinical outcomes in acute ICH.

Methods: INTERACT2 was an international, open, blinded endpoint, randomized controlled trial. Eligible patients with spontaneous ICH within 6 h of onset and elevated systolic BP (SBP; 150–220 mmHg) were allocated to receive intensive target SBP <140 mmHg within 1 h using intravenous agents) or guideline-recommended (SBP <180 mmHg) BP lowering treatment. BP reduction was defined as baseline SBP minus average of achieved SBP levels during 3 periods after randomization (15–60 minutes, 1–24 hours, and 2–7 days). Outcome was death or major disability at 90 days.

Results: Larger reductions in SBP between 15 and 60 minutes were associated with lower risks of death or major disability (P trend <0.01). Similar associations were also observed for SBP reductions between 1 and 24 h (P trend <0.01) and between 2 and 7 days (P trend =0.01). The association of SBP reductions and death or major disability appeared to be stronger among patients with baseline SBP levels of ≥180 mmHg than those with baseline SBP <180 mmHg (P homogeneity=0.07 for 15–60 minutes, 0.04 for 1–24 hours and 0.02 for 2–7 days).

Conclusion: Optimal protection against death or major disability after ICH was observed in patients who achieved the greatest SBP reductions in the first hour and maintained these consistently over 7 days.

H-040

COST EFFECTIVENESS OF HYPERTENSION MANAGEMENT GUIDED BY CENTRAL BLOOD PRESSURE MEASUREMENT: HEALTH ECONOMIC EVALUATION OF THE BP GUIDE STUDY

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Background: The BP GUIDE study was a clinical trial in 286 hypertensive patients randomized to treatment decisions guided by best-practice usual care or in addition by central BP measurement in order to gauge financial viability. Health system savings were calculated based on capital costs of $10,000 (for SphygmoCor), 5 years capital life, 5% discount rate, and increased SBP reductions between 1 and 24

H-041

ALDOSTERONE-INDUCED OXIDATIVE STRESS AND INFLAMMATION IN THE BRAIN: ROLE OF THE ENDOTHELIAL CELL MINERALOCORTICOID RECEPTOR

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Background: Elevated aldosterone levels are associated with stroke risk that is independent of blood pressure and other risk factors. Aldosterone acts on the mineralocorticoid receptor (MR) in the kidney to regulate fluid and electrolyte homeostasis and blood pressure, but can also act on other tissues such as the brain and its vasculature, and cause detrimental effects such as oxidative stress and inflammation.

Aim: To examine whether aldosterone causes endothelial cell MR-dependent oxidative stress in cerebral vessels, and increases pro-inflammatory markers in the brain.

Methods: Male mice were anesthetized with ketamine (100 mg/kg)/xylazine (10 mg/kg) i.p. and treated with vehicle or aldosterone (0.28 or 0.72 mg/kg/day) by osmotic minipumps for 2 weeks. Endothelial cell MR-deficient (MR<sup>−/−</sup>/Te2<sup>−/−</sup>) and wild-type (MR<sup>+</sup>/Te<sup>+</sup>) mice, and C57Bl/6 mice pre-treated with spironolactone (25 mg/kg/day, i.p.), an MR antagonist, were used for tests to fMRI evokement. Systemic blood pressure (SBP) was measured using tail cuff plethysmography, superoxide levels using L012 chemiluminescence, and chemokine ligand (CCL7, CCL12) and receptor (CCR2) mRNA expression using real-time PCR.

Results: In C57Bl/6 mice, aldosterone (0.72 mg/kg/day) moderately increased SBP when compared to vehicle (SBP, vehicle-treated=102 mmHg; n=10; SBP, aldosterone-treated=16.32 mmHg; n=11, P<0.05). No aldosterone-dependent increases in superoxide were ~60% greater in cerebral arteries from aldosterone- vs vehicle-treated mice (n=7–8; P<0.05). Pretreatment with spironolactone prevented aldosterone-induced increases in Nox2-dependent superoxide (n=5; P>0.05), suggesting this effect was MR-dependent. In wild-type (MR<sup>+</sup>/Te<sup>+</sup>) mice, Nox2-dependent increases in superoxide were ~50% greater in cerebral arteries from aldosterone- vs vehicle-treated mice (n=7–8; P<0.05), and this effect was abolished in endothelial cell MR-deficient (MR<sup>−/−</sup>/Te2<sup>−/−</sup>) mice (n=7–8; P>0.05). Aldosterone increased expression of CCL7 mRNA in the brain compared to ~1.8-fold of vehicle, (n=11–12; P=0.07), but had no effect on CCL12 or CCR2 mRNA expression (n=11–12; P=0.13 and P=0.14, respectively).

Conclusion: Chronic aldosterone administration increases cerebrovascular superoxide levels by a mechanism involving endothelial cell MR. This treatment also increases expression of CCL7 mRNA in brain. Endothelial cell-specific MR antagonism may represent a novel approach to treat cerebrovascular disease.

H-042

EXERCISE-INDUCED ALBUMINURIA IS INDEPENDENTLY RELATED TO EXERCISE AORTIC RE_REPOUS T FUNCTION IN PATIENTS WITH TYPE 2 DIABETES

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Background: Patients with type 2 diabetes (T2D) are susceptible to exercise-induced albuminuria even at submaximal exercise, but the mechanisms are unknown. Recent data indicates that T2D patients have raised central blood pressure (BP) during submaximal exercise and this could contribute to renal dysfunction independent of upper arm BP.

Aim: To determine the relationship between exercise central hemodynamics and exercise-induced albuminuria in T2D.

Methods: Forty T2D patients (aged 62±9 years; 50% male) and 40 healthy controls (aged 55±9 years; 50% male) were examined at rest and during a 20 minute bout of low light cycle exercise (40W, 50rpm). Hemodynamics recorded included aortic reservoir function (excess pressure integral (xSP) and aortic reservoir pressure), aortic stiffness, augmented pressure (AP), brachial and central BP. Albuminuria was assessed by albumin/creatinine ratio (ACR) at rest and within 20 minutes after exercise.

Results: There was no difference between groups in resting ACR (P>0.05). Exercise induced a significant rise in ACR in T2D patients but not controls (0.39±0.89 vs 1.05±1.38 mEq/ml; P=0.017). All central hemodynamic variables indicative of systolic stress were significantly higher during exercise in T2D participants (i.e., xSP, systolic BP and AP; all P<0.01). For T2D patients, exercise xSP was associated with increased ACR (β=0.003; P=0.001), independent of age, sex, body mass index, and 24 hour ambulatory SBP.

Conclusion: Aortic reservoir function, as determined by excess pressure during submaximal exercise, is independently associated with exercise-induced albuminuria in T2D patients. These novel findings suggest that aortic reservoir function could be important for appropriate renal function in patients with T2D.

H-043

INFAMMASOME ACTIVITY IS ESSENTIAL FOR DEOXYCORTICOSTEROSE ACETATE/SALT-INDUCED HYPERTENSION IN MICE


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Background: Inflammammosomes are signaling complexes comprised of a NOD-like receptor protein (NLRP), an adapter protein (ASC) and caspase-1. Inflammammosomes detect host-derived danger signals and cause an inflammatory response via activation of caspase-1, which in turn processes the pro-inflammatory cytokines pro-interleukin (IL)-1β and pro-IL-1β into their active forms. Hypertension is associated with chronic renal inflammation, but the role of the inflammasome in this inflammatory process has not been assessed.

Aim: To investigate whether hypertension in mice is associated with increased expression and activation of the inflammasome and to assess the impact of genetic inhibition of the inflammasome on blood pressure (BP) and renal hypertension during hypertension.

Methods: Male C57Bl/6J (wild type), NLPRF<sup>−/−</sup> and ASC<sup>−/−</sup> mice aged 10–12 weeks had their left kidney removed but received a placebo pellet and normal drinking water.
water (1K/placebo). Tail cuff plethysmography was used to monitor systolic BP. After 21 days, mice were killed via isoflurane inhalation and the remaining kidney was excised for determination of mRNA for NLRP3, ASC, caspase-1, pro-IL-1β, and pro-IL-18, along with markers of renal inflammation (IL-6, IFN-γ, COL2.1, CCL5, iC3M, VCAM1) by real-time PCR. Inflammase activation was assessed by Western blotting for the active caspase-1 p10 subunit (10kDa).

Results: 1K/DOCA/salt-treated mice had elevated systolic BP (147 ± 4 mmHg) compared to 1K/salt-treated mice (123 ±13; P=0.05). Expression of NLRP3, ASC, caspase-1 and pro-IL-1β mRNA and protein expression of activated caspase-1 were all significantly increased in kidneys of 1K/DOCA/-salt- versus 1K/placebo-treated mice. Inflammasome-deficient NLRP3–/– and ASC–/– mice displayed blunted hypertensive responses to 1K/DOCA/salt-treatment (149 ± 4 and 141 ± 4 mmHg, respectively) compared to wild-type (165 ± 5 mmHg; n=25; P<0.05). Surprisingly, this reduction in BP was independent of an effect on inflammasome-deficiency on expression of inflammasome components or renal inflammatory markers.

Conclusion: 1K/DOCA/salt hypertension in mice is critically dependent on a functional inflammasome, although the mechanism by which inflammasome activity regulates BP remains to be determined.

POTENTIAL VASCULAR MECHANISMS OF RAMIPRIL-INDUCED INCREASES IN WALKING ABILITY IN PATIENTS WITH INTERMITTENT CLAUDICATION

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Aim: To conduct exploratory analyses of the effects of ramipril therapy on circulating biomarkers of angiogenesis/arteriogenesis, thrombosis, inflammation and leukocyte adhesion in patients with intermittent claudication.

Methods: 165 patients with intermittent claudiction (65.3 ± 6.75 years), administered ramipril 10 mg/day (n=82) or matching placebo (n=83) for 24 weeks, in a randomized, double-blind, placebo-controlled study. Plasma biomarkers of angiogenesis/arteriogenesis – vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF2), thrombosis (D-dimer), von Willebrand Factor (vWF); thrombin-antithrombin III (TAT), inflammation (high sensitivity C-reactive protein (hsCRP)); osteopontin (OPN); and leukocyte adhesion (soluble vascular cell adhesion molecule-1 (sVCAM-1); soluble intracellular adhesion molecule-1 (sICAM-1)) were measured at baseline and at 24 weeks.

Results: Relative to placebo, ramipril was associated with increases in VEGF by 38% (95% CI, 27–50), OPN by 12% (95% CI, 3–20), sVCAM-1 by 14% (95% CI, 4–24), sICAM-1 by 15% (95% CI, 4–26), and P-selectin by 20% (95% CI, 9–31).

Conclusions: Ramipril is associated with an increase in biomarkers of angiogenesis/arteriogenesis and reduction in markers of thrombosis, inflammation and leukocyte adhesion. This study informs strategies to improve mobility in patients with intermittent claudication.

IMPLICATIONS OF DIET MODIFICATION ON SYMPATHOINHIBITORY MECHANISMS AND HYPERTENSION IN OBESITY

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Background: Over 50% of cardiac output is directed towards the gut and kidney postprandially, highlighting the importance of blood flow regulation to these regions. The gastrointestinal hormone cholecystokinin (CCK) acts at vagal afferents to induce renal and splanchnic sympathoinhibition and vasodilation, via reflex inhibition of a subclass of cardiovascular-controlling neurons in the rostroventrolateral medulla. We have demonstrated that sympathetic inhibition, vasodilator and central neuronal responses to CCK are blunted in obese hypertensive rats fed a moderately high fat diet (MHFD), possibly impacting on cardiovascular homeostatic mechanisms and contributing to the etiology of obesity-related hypertension.

Aim: To determine whether swapping a MHFD for a low fat diet (LFD) would reverse the signs of hypertension and restore sympathoinhibitory reflexes in obese hypertensive rats.

Methods: Male Sprague-Dawley rats were placed on a LFD (control; n=8) or a MHFD (n=24) for 11 weeks; the latter animals exhibited either an obesity-prone (OP) or obesity-resistant (OR) phenotype as determined by weight gain falling into the upper or lower tertile, respectively. All animals were then placed on the LFD for a further 6 week period after which they were anaesthetised with isoflurane and cannulated for evaluation of resting arterial pressure (AP) and renal nerve responses to CCK (0.1–4 μg/kg).

Results: Weight gain in OP animals remained higher than OR or controls following diet switch (P<0.05 for both). Resting AP was not significantly different between OP (103 ± 4 mmHg), OR (102 ±3 mmHg) and controls (104 ±3 mmHg) and sympathoinhibitory responses to CCK were not significantly different between the groups (P>0.05 for all concentrations).

Conclusion: These results further implicate sympathoinhibitory mechanisms in the etiology of obesity-related hypertension, and demonstrate that diet modification can have beneficial effects on sympathetic function and restore normotension without the need for weight reduction.

VASCULAR ENDOTHELIAL GROWTH FACTOR-MEDIATED SIGNALOSOME FORMATION AND S-NITROSYLATION OF CELL CYCLE-RELATED PROTEINS IN PROLIFERATING HUMAN ENDOTHELIAL CELLS

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Background: In previous studies we established the role played by the VEGF/Akt1/eNOS/NO signaling pathway in mediating cell proliferation of angioblasts and embryonic endothelial cells (ECs) during murine development (Gentile et al., Dev Biol 2013;373:163–175). These findings, together with the emerging role(s) played by HSP90 in mediating the formation of the signalosome (the multimolecular complex comprising pAkt1, pECS1, HSP90 and the molecular target of NO) in human endothelial cells, led us to investigate the relevancy of the VEGF/eNOS signaling pathway in human ECs.

Aim: To investigate the role(s) played by the VEGF/eNOS signaling pathway in mediating signalosome formation in proliferating human ECs.

Methods: Levels of eNOS phosphorylation at S117 (peNOS) were evaluated by western blot analysis in serum-starved (SS) human umbilical vein endothelial cells (HUVECs) and HUEVECs synchronized in the G2/M phase by the addition of nocodazole. We also compared proliferation between HUEVECs overexpressing wild type eNOS (eNOS HUEVECs) and HUEVECs that express a mutated, unphosphorylatable eNOS (eNOS S117C/HUEVECs).

Results: peNOS levels were increased in mitotic HUEVECs compared to SS HUEVECs. An increase in proliferation observed in eNOS HUEVECs compared to HUEVECs overexpressing a scrambled plasmid was dependent on the phosphorylation of eNOS on Ser117. Furthermore, VEGF-induced eNOS-S-nitrosylation of cyclin B1, controlling the G2/M transition in HUEVECs by western blot analysis. There was a correlation between HSP90 binding and S-nitrosylation of cyclin B1 following VEGF treatment. Finally, VEGF-mediated S-nitrosylation of cyclin B1 was substantially reduced in the presence of A-73, an inhibitor of HSP90, demonstrating that the VEGF-mediated S-nitrosylation of cyclin B1 is dependent on HSP90 in human ECs.

Conclusions: These studies have demonstrated for the first time that VEGF-mediated proliferation in human ECs is dependent on the S-nitrosylation of cyclin B1 following signalosome formation. Current studies are focusing on evaluating whether VEGF mediates proliferation via S-nitrosylation of cell cycle-related proteins in other cell types, such as cardiomyocytes.
to pharmacological manipulation of peripheral resistance. Another significant result was the augmentation of baroreceptor sensitivity during stimulation.

Background: Stem cell-based therapies to repair and regenerate lost myocardium have potential to revolutionize modern medicine for treatment of myocardial infarction and heart failure. We have recently identified a novel subclass of human cardiac resident stem cells (CRSCs) that are positive for WBB2 antigen.

Aim: To characterize the human WBB2+ CRSCs isolated from adult human atrial appendages.

Methods: WBB2+ CRSCs were isolated from explants of human atrial appendages. Cells were screened by flow cytometry for expression of Islet-1 and Nkx2.5, and further characterized using immunocytochemistry and gene expression profiling.

Results: Human WBB2+ CRSCs can self-renew (population doubling time of ~27 hours) and are highly clonogenic (cloning efficiency of 50%). Immunophenotyping showed they were CD29+ (100%), CD73+ (87.4%), CD90+ (65.7%), CD105+ (86.8%), HLA-ABC (83.3%), were largely negative for HLA-DR (0.03%), CD31+ (1.7%), CD34+ (1.3%), CD45+ (9.2%), CD133+ (0.2%), and Lin+ (0.8%) (n=4). RT-PCR analysis of these cells failed to detect T-box1 or Nkx2.5 mRNA expression, and immunostaining showed that WBB2+ CRSCs expressed GATA4, GATA7, vimentin and nestin, but not Wnt’s tumour gene-1 (a specific marker of epidermal progenitors) or discoidin domain receptor-2 (a specific marker of fibroblasts). Of 274 human cytokerins analysed, WBB2+ CRSCs were found to secrete various cytokines implicated in angiogenesis (angiogenin, angiopoietin, bFGF), chemokias (MCP-1, eotaxin, RANTES, etc), inflammation (IL-6, GDF-15, etc), extracellular matrix remodelling (decorin, MMP-10, etc), growth and survival (DPP-4, HGF, follistatin, etc). WBB2+ CRSCs can differentiate into cardiogenic cells that are responsive to electrical stimulation, into endothelial and smooth muscle cells, as well as being able to undergo adipogenesis, osteogenesis and chondrogenesis. WBB2+ CRSCs survived through in vivo implantation and were found to occupy the peripheral spaces.

Conclusion: WBB2+ CRSCs are distinct from currently known CRSCs found in human heart. WBB2+ CRSCs may be an important cardiovascular cell source for tissue engineering and for autologous cell therapies in patients with cardiovascular diseases.

RAISED SOLUBLE P-SELECTIN MODULATES PLAQUE INSTABILITY IN AN EXPERIMENTAL MODEL OF VULNERABLE PLAQUE


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Background: We have previously shown that sP-selectin increases leukocyte recruitment to immobilized platelets and the resting endothelium. We therefore hypothesize that chronically raised sP-selectin will hasten the progression of atherosclerosis in a genetically susceptible mouse model of the disease, the SM22α-nTDR-Apo ER mouse.

Aim: To determine whether sP-selectin alters plaque phenotype in SM22α-nTDR-Apo ER mice.

Methods: SM22α-nTDR-Apo ER mice and Apo ER littermate controls were placed on a high fat diet (30% fat) and treated with recombinant murine sP-selectin (150 ng/kg/day for each administered s.c. by daily injections). Five ng/ml of diphtheria toxin was injected 3 times per week from week 12 to 16 to induce VSMC apoptosis. Six-micrometer cross-sections of aortic sinus were examined for macrophage and leukocyte accumulation via CD68 and CD45, respectively, lipid deposition via oil red O and collagen content via picrosirius red staining. sP-selectin concentration was analysed for cytokine/chemokine content.

Results: Plasma analysis indicated that sP-selectin treatment was associated with: significantly higher macrophage accumulation (P-selectin; 11.7 ± 2.35%; sP-selectin; 5.0 ± 1.07%; n=6–8; P<0.01), significantly higher CD45 content (P-selectin: 9.3 ± 1.4%; sP-selectin: 8.8 ± 0.52%; n=7–8; P<0.03) and significantly lower collagen content (sP-selectin: 5.8 ± 0.72 % vs sP-selectin: 11.1 ± 1.7%; n=5–8; P<0.04). Lipid deposition was not significantly different between groups. After 16 weeks of treatment, plasma MCP-1 and RANTES were increased and decreased, respectively, in sP-selectin treated mice.

Conclusion: These results suggest that chronically raised sP-selectin favours progression of an unstable atherosclerotic plaque phenotype.

T CELL INFLTRATION IN THE AORTA AND KIDNEY OF HYPERTENSIVE RATS IS Y CHROMOSOME DEPENDENT

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Background: The immune system is essential for the development of hypertension. We have shown whether the Y chromosome from a hypertensive or normotensive rat strain accounts for 12–15 mmHg difference in blood pressure. The copy of the Y chromosome in humans associated with increased risk of CVD is also associated with an increased regulation of inflammatory gene expression, suggesting that the origin of the Y chromosome may influence CVD risk by augmentation of the immune response. Whether the origin of the Y chromosome directly influences immune responses in the context of hypertension, remains unknown.

Aim: To examine the influence of the origin of the Y chromosome on immune cell infiltration into aortic and kidney tissue.

Methods: Male rats were crossed with WBB2+ CRSCs isolated from adult human atrial appendages. Cells were screened by flow cytometry for expression of Islet-1 and Nkx2.5, and further characterized using immunocytochemistry and gene expression profiling.

Results: Human WBB2+ CRSCs can self-renew (population doubling time of ~27 hours) and are highly clonogenic (cloning efficiency of 50%). Immunophenotyping showed they were CD29+ (100%), CD73+ (87.4%), CD90+ (65.7%), CD105+ (86.8%), HLA-ABC (83.3%), were largely negative for HLA-DR (0.03%), CD31+ (1.7%), CD34+ (1.3%), CD45+ (9.2%), CD133+ (0.2%), and Lin+ (0.8%) (n=4). RT-PCR analysis of these cells failed to detect T-box1 or Nkx2.5 mRNA expression, and immunostaining showed that WBB2+ CRSCs expressed GATA4, GATA7, vimentin and nestin, but not Wnt’s tumour gene-1 (a specific marker of epidermal progenitors) or discoidin domain receptor-2 (a specific marker of fibroblasts). Of 274 human cytokerins analysed, WBB2+ CRSCs were found to secrete various cytokines implicated in angiogenesis (angiogenin, angiopoietin, bFGF), chemokias (MCP-1, eotaxin, RANTES, etc), inflammation (IL-6, GDF-15, etc), extracellular matrix remodelling (decorin, MMP-10, etc), growth and survival (DPP-4, HGF, follistatin, etc). WBB2+ CRSCs can differentiate into cardiogenic cells that are responsive to electrical stimulation, into endothelial and smooth muscle cells, as well as being able to undergo adipogenesis, osteogenesis and chondrogenesis. WBB2+ CRSCs survived through in vivo implantation and were found to occupy the peripheral spaces.

Conclusion: WBB2+ CRSCs are distinct from currently known CRSCs found in human heart. WBB2+ CRSCs may be an important cardiovascular cell source for tissue engineering and for autologous cell therapies in patients with cardiovascular diseases.
A CR2 RECEPTOR ANTAGONIST, INCB3344, REDUCES MACROPHAGE ACCUMULATION AND AORTIC FIBROSIS AND LOWERS BLOOD PRESSURE IN ANGIOTEIN I INHIBITION IN MICE


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Background: Vascular fibrosis and stenosing are hallmarks of hypertension and contrib-
ite to elevated systolic blood pressure (SBP) and end organ damage. Hypertension is also
associated with macrophage accumulation in the vessel wall. Macrophages are attracted into tissues by chemokines and can adopt pro-inflammatory (M1) or anti-inflammatory/pro-
fibrotic (M2) phenotypes. Thus, macrophages could conceivably contribute to the vascular
tissues that occur in hypertension.

Aim: To determine the polarization state of macrophages in the vessel wall during hypertension
and to investigate if pharmacological antagonism of the chemokine receptor-ligand
interactions responsible for their accumulation reduces vascular fibrosis, and stenosing,
and SBP in hypertension.

Methods: Angiotensin II (Ang II) (0.7 mg/kg/day, s.c.) was infused for 28 days into male C57BL6/J
mice. Macrophage markers were measured by flow cytometry and real-time PCR. The effect of a
CR2 antagonist, INCB3344 (30 mg/kg, i.p.), on the response to Ang II was examined.

Results: Ang II infusion caused a sustained increase in SBP (138 ± 3 mmHg) compared to
saline-infusion (122 ± 3 mmHg; n=24, P<0.0001). There was a 2-fold increase in total macro-
phage (F4/80+) numbers in Ang II-infused mice. These cells were M2 polarized based on
expression of the M2 marker, CD206. Real-time PCR confirmed that CD206 expression was
upregulated by 55% (n=5; P<0.05). We observed a greater number of infiltrated T helper cells in the aorta
and a greater infiltration of T helper cells, cytotoxic T cells and regulatory T cells in the kid-
ney. Replacement of the hypertensive Y chromosome with the normotensive Y chromosome
(SPWKY/ Y) significantly reduced T cell infiltration to WKY levels in the aorta and kidney.
There were no differences in circulating plasma or splenic immune cell levels between any
of the strains, suggesting that the origin of the Y chromosome influences T cell function and
infiltration rather than production.

Conclusion: Our data demonstrates that the increased T cell infiltration observed in hypertension
rats in target cardiovascular organs, such as the kidney and aorta, is Y chro-
mosome-dependent. We suggest that there are genes located on the Y chromosome that
influence blood pressure regulation via immunological responses and that they may predispose certain males to the development of CVD.

H-058

HUMAN TOR COMPLEX COMPONENTS IN GENETICS OF LONGEVITY AND HYPERTENSION


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Background: Evolutionarily conserved nutrient/energy-sensing pathways influence com-
mon conditions of aging and lifespan. Each of these likely invoke the hyperfunction theory
of aging. Mechanistic target of rapamycin (mTOR) is at the hub of one such pathway. mTOR
inhibition extends mammalian lifespan. mTOR is a critical regulator of cardiac hypertrophy
in the SHR. Although genetic factors play major roles in chronic conditions of aging and
attainment of extreme old age, few genes have been implicated.

Aim: To test tagging single nucleotide polymorphisms (tagSNPs) that captured most of the
genetic variation across the key TOR complex 1 (TORC1) and TOR complex 2 (TORC2) genes
MTOR, RPTOR and RICTOR and the important downstream effector gene RPS6KA1 for
association with human longevity (defined as attainment of at least 95 years of age) and
clinically relevant phenotypes of aging.

Methods: Subjects comprised a homogeneous population of American men of Japanese
ancestry, well characterized for aging phenotypes and that have been followed for 48 years.
We used a nested-case control study design involving 440 subjects aged ≥85 years (range
95–106) and 374 controls (age at death 73–81). Genotypes were determined for 6 tagging
SNPs for MTOR, 61 for RPTOR, 7 for RICTOR, as well as 5 for RPS6KA1. Associations were
examined for 40 phenotypes. Analyses involved χ2 and ANOVA.

Results: Data showed long-lived cases were healthier than controls, especially after adjust-
ing for age. They had lower blood pressure and less hypertension at age 71–93 years. There
was no significant association of any of the 79 SNPs with longevity. Phenotypes related to
blood pressure, essential hypertension (EHT), isolated systolic hypertension (ISH) and
body weight showed genotypic associations with 23 tagSNPs of RPTOR. The associations
remained after correction for multiple testing.

Conclusion: TOR complex genes showed no genetic association with longevity, but an
association of RPTOR SNPs with hypertension and related phenotypes was apparent in

H-061

A QUANTITATIVE ANALYSIS OF THE FACTORS INFLUENCING OXYGEN DIFFUSION IN THE VICINITY OF ARTERY-VEIN PAIRS IN THE KIDNEY


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Background: Diffusion of oxygen from arteries to veins in the kidney (AV oxygen shunting)
acts to limit oxygen delivery to renal tissue. We recently employed computational modeling
to identify two factors critical to determination of the quantity of AV oxygen shunting within
the renal circulation. These were (i) the distance between the artery and the vein, and (ii)
the angle between the vein and the wall of the renal circulation.

Aim: To quantify how the factors in (i) and (ii) above change along the course of the renal circulation.
Methods: The renal vasculature of Sprague Dawley rats (n=6) was perfusion fixed and filled with Microfil®. A section from each kidney was chosen and the shortest arterial/arteriolar diameter, distance to the nearest vein, and the degree to which a vein wraps an artery were measured.

Results: The diffusion distance between arteries and veins increased with decreasing arterial diameter (Figure 1). The proportion of the arterial wall surrounded by the vein (wrapping) decreased as arterial diameter decreased (Figure 2).

Conclusions: The spatial relationships (separation and wrapping) between arteries and veins that promote AV oxygen shunting are more prominent in the larger vessels than the smaller vessels of the kidney. These observations challenge the conventional notion that most AV oxygen shunting occurs in the smaller cortical vessels (e.g., interlobular arteries) after the divergence of the cortical and medulary circulations. Thus, AV oxygen shunting may limit oxygen delivery to the renal medulla as well as the renal cortex.

BLOOD-BRAIN BARRIER LEAKAGE IN THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS OF RENOVASCULAR HYPERTENSIVE RATS

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Background: An intact blood-brain barrier (BBB) is important for normal brain function. Whilst the integrity of the BBB is thought to be affected in a number of diseases, its permeability has not yet been investigated in hypertensive animals.

Aim: To evaluate whether, in an angiotensin II-dependent model of hypertension, two-kidney one clip (2K1C) hypertension, the BBB is disrupted in the paraventricular nucleus of the hypothalamus (PVN), a nucleus that plays a central role in cardiovascular regulation.

Methods: Renovascular hypertension was induced by placing a constricting clip around the kidney one clip (2K1C) hypertension, the BBB is disrupted in the paraventricular nucleus of the hypothalamus (PVN), a nucleus that plays a central role in cardiovascular regulation.

Results: Hypertensive rats (systolic blood pressure 188 ± 4 vs. control 109 ± 5 mmHg; P<0.0001) showed greater EB leakage in the PVN compared with control normotensive rats. EB intensity was 31% higher in the paraventricular subdivision (P<0.05) and 39% higher in the magnocellular subdivision (P<0.05) of the PVN in the 2K1C group. No significant change of EB intensity was, however, found in the cortex.

Conclusion: Our data show that in the PVN of 2K1C rats the BBB is compromised, suggesting that in hypertensive states this cardiovascular region might be susceptible to the actions of substances that do not normally cross the BBB.

RENAL TISSUE HYPOXIA IN A RAT MODEL OF POLYCYSTIC KIDNEY DISEASE

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Background: Polycystic kidney disease (PKD) is characterized by the development of numerous fluid-filled cysts. Hypoxia has been identified as a final common pathway in the progression of chronic kidney disease. Histological analysis of kidney sections from animals with PKD have provided qualitative evidence of tissue hypoxia. To date, no direct measurements of tissue PO2 have been made.

Aim: To directly measure renal tissue PO2 in the Lewis rat model of PKD (LPK) and to determine the relative contributions of altered renal oxygen delivery and oxygen consumption in driving tissue hypoxia.

Methods: Experiments were performed in 11–13 week-old Lewis and LPK rats. Rats were anesthetized with sodium thiobarbital and artificially ventilated. Renal tissue oxygenation was measured using the Clark electrode (10 µm diameter). Tissue PO2 was determined within multiple sites in the renal parenchyma and in cysts in the superficial cortex (n=12 Lewis; n=11 LPK). Arterial and renal venous oxygen content were determined by direct blood oximetry.

Results: In LPK rats, tissue PO2 was higher within the cysts (32.8 ± 4.0 mmHg) than in the superficial cortical tissue itself (18.3 ± 3.5 mmHg), but still lower than the tissue PO2 of the superficial cortex of Lewis rats (46.0 ± 3.1 mmHg). Renal tissue oxygen delivery was 78.5% lower in LPK rats than Lewis rats. Total sodium reabsorption was 88.0% less in LPK rats than Lewis rats, but renal oxygen consumption did not differ significantly between LPK and Lewis rats.

Conclusion: In this model of PKD, the superficial renal cortex is severely hypoxic. Tissue hypoxia in the kidney of LPK rats is driven in part by the compromised tissue oxygen delivery. Our inability to detect a significant deficit in renal oxygen consumption in LPK rats, despite a marked deficit in sodium reabsorption, suggests that inefficient utilization of oxygen for sodium reabsorption may also contribute to the development of renal hypoxia in PKD.

BLOOD PRESSURE VARIATION AND END ORGAN DAMAGE IN PATIENTS WITH UNCOMPlicated HYPERTENSION: INFLUENCE OF ANThYHYPERTENSIVE MEDICATION TITRATION

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Background: There are limited data regarding effects of antihypertensive medication titration on blood pressure variation (BPV).

Aim: To investigate the effect of antihypertensive medication titration on BPV and the relation to end organ damage associated with hypertension.

Methods: Data were analysed from 218 patients with uncomplicated hypertension (age 64 ± 8; males 54%) who participated in a clinical trial over 12 months in which doses of antihypertensive medication were altered but BP remained controlled. Patients were stratified based on decreasing (n=68), maintained (n=88) or increasing (n=35) medication over time. Daytime systolic BP variation (BPV) was calculated from 24-hour ambulatory BP. Left ventricular mass index (LVMi) was derived from three-dimensional echocardiography. Changes in BPV and LVMi were calculated by the difference between baseline and 12-month measurements.

Results: Increasing medication reduced BPV compared with steady state or decreasing medication (8.3 ± 15.2 vs –1.1 ± 13.7 and –1.6 ± 14.5 mmHg/h, respectively; P<0.05), but no significant change in LVMi was observed between groups. The change in BPV was significantly related to the change in LVMi only in the group with decreasing medication (n=302; P=0.01). This relationship was maintained after multiple regression analysis adjusting for age, body mass index and sex (unstandardized β =0.062; P=0.018).

Conclusions: Changes in antihypertensive medications appear to have differential effects on BPV, as well as the relationship between BPV and end organ damage. These findings have potential implications regarding appropriate medical therapy of patients with uncomplicated hypertension.

ROLE OF ANDROGENS IN SEX DIFFERENCES IN CARDIAC DAMAGE DURING MYOCARDIAL INFARCTION

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Background: Age-specific incidence of ischemic heart disease in men is higher than in women. The molecular mechanism(s) are poorly understood since most studies focus on estradiol (E2) actions mediated via estrogen receptors (ER), with less attention to androgen receptor (AR)-mediated androgen actions. We have previously reported larger infarct...
size and aggravated apoptosis in hearts from intact males than female hearts following ischemia-reperfusion injury (MI) in both males and females.

Methods: Mature age-matched male and female Sprague Dawley rats – intact or surgically gonadectomized (Gx) – received testosterone (T) or estradiol (E2) via subdermal silastic implants. A subset of male rats received dihydrotestosterone (DHT). After 21 days, hearts were subjected to ex vivo regional I-R.

Results: In Gx males, androgens (DHT, T) and E2 aggravated I-R induced cardiac damage, whereas in Gx females, T had no effect and E2 reduced infarct area. Increased circulating tissue levels upregulated AR and receptor for advanced glycation end products (RAGE), aggravating cardiac damage in both males and females by preventing progression of autophagy and decreasing levels of anti-apoptotic proteins.

Conclusions: We demonstrate a novel and key role for testosterone during myocardial I-R to increase levels of androgen receptors and RAGE, which contributes to aggravated cardiac damage in both male and female gonadectomized rats. Our results provide a new platform for potential new treatment strategies for reperfusion injury in both males and females.

H-066
LEPTIN REDUCES FOOD INTAKE BUT FAILS TO RAISE BLOOD PRESSURE IN MICE WITH DEFICIENCY OF INSULIN RECEPTOR SUBSTRATE (IRS2) IN THE ENTIRE BRAIN OR SPECIFICALLY IN POMC NEURONS

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Background: Insulin receptor substrate (IRS2) is one of three major insulin receptor signaling
pathways. Its role in mediating the chronic effects of leptin on appetite, blood pressure and glucose regulation is, however, unclear.

Aim: To test whether deletion of IRS2 in the whole brain or specifically in proopiomelanocor-
tin (POMC) neurons attenuates the chronic cardiovascular and metabolic responses to leptin.

Methods: Mice with IRS2 deletion in the entire brain (IR32mfn/Nestin-Cre; n=7), specifically in POMC neurons (IR32mfn/Pomc-Cre; n=7) and control IRS2mfn/+ (n=9) mice were instrumented with telemetry probes for measurement of 24 hour mean arterial pressure (MAP) and heart rate (HR) and venous catheters for saline or leptin infusions. After a 5-day control period, mice received leptin infusion (2 µg/kg/min, i.v.) for 7 days.

Results: Compared to IRS2mfn/+ mice, IRS2mfn/Pomc-Cre mice had similar body weight and food intake (33 ± 1 vs 35.1 ± 3.6 g and 0.5 ± 3.8 ± 2.0 g/day) and higher MAP and HR (110 ± 2 vs 102 ± 2 mmHg and 641 ± 9 vs 616 ± 5 bpm). IRS2mfn/Nestin-Cre mice were heavier (38 ± 1.5 g), had increased food intake (4.5 ± 1.0 g/day), and higher MAP and HR (108 ± 1.5 mmHg and 659 ± 9.0 bpm) compared to control mice. Leptin infusion for 7 days in control IRS2mfn/+ mice gradually increased MAP by 5 mmHg despite decreasing food intake by 31%. In contrast, leptin infusion did not change MAP in IRS2mfn/Nestin-Cre and IRS2mfn/Pomc-Cre mice. The anorexic effect of leptin was not, however, attenuated in IRS2mfn/Pomc-Cre or IRS2mfn/Nestin-Cre mice (n=35% and 34%, respectively). In addition, deletion of IRS2 in the whole brain or POMC neurons did not impair the ability of leptin to reduce plasma glucose levels (IR32mfn/+ mice; 170 ± 15 to 130 ± 12; IRS2mfn/Pomc-Cre; 180 ± 20 to 150 ± 15; IRS2mfn/Nestin-Cre; 160 ± 10 to 131 ± 20 mg/dl).

Conclusion: These results indicate that activation of IRS2 signaling in the CNS, and particularly in POMC neurons, is essential for the long-term actions of leptin to raise MAP and HR, but not for its anorexic or antidiabetic effects.

H-067
THE EFFECT OF CHRONIC ANGIOTENSIN TYPE 2 RECEPTOR STIMULATION ON THE ADHESION CASCADE AND PLAQUE DEPOSITION IN APOE–/– MICE FED A HIGH FAT DIET


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Background: The angiotensin type 1 receptor (AT1R) stimulates vasoconstrictive, pro-fibrotic and pro-inflammatory effects to the endothelium, is a critical early event in the development of atherosclerosis. Activation of IRS2 signaling in the CNS, and particularly in POMC neurons, is essential for the long-term actions of leptin to raise MAP and HR, but not for its anorexic or antidiabetic effects.

Aim: To assess relevant features of AAA induced by calcium phosphate in a mouse model.

Methods: AAA was induced by perivascular application of calcium phosphate to the infrarenal aorta of 3 and 7 month old male mice. Infra-renal aortic diameter was assessed prior to AAA induction and 2 weeks later. AAA induction was assessed by calculating expansion of the infra-renal aortic diameter over 2 weeks. Blood pressure was measured by the tail cuff method, and plasma concentrations of total cholesterol, low density lipoprotein and very low density lipoprotein cholesterol, pro-inflammatory cytokines and matrix metalloproteinase-9 were measured using commercially available kits.

Results: The median expansion of the infra-renal aorta 2 weeks after AAA induction was significantly greater in mice that were deficient in apolipoprotein E than in the age- and gender-matched wild-type controls (276% versus 94.7%; P=0.02). Plasma low density lipoprotein/very low density lipoprotein cholesterol concentrations 2 weeks after AAA induction were positively correlated with the expansion of the infra-renal aorta induced by calcium phosphate (Spearman r=0.661; P=0.04). The median expansion of the infra-renal aorta 2 weeks after AAA induction was similar in 3 and 7 month-old wild-type mice (121% versus 126%; P=0.33). The local administration of calcium phosphate was associated with an increase in the mean maximal diameter of distant aortic segments, but not associated with changes in the concentrations of circulating pro-inflammatory markers.

Influence of Apolipoprotein E, Age and Aortic Site on Calcium Phosphate Induced Atherosclerotic Aneurysm in Mice

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Background: Abdominal aortic aneurysm (AAA) affects –5% men aged >65 years and is an important cause of morbidity and mortality. The study of AAA pathogenesis in humans is limited. The use of appropriate animal models could potentially have an important role in furthering the understanding of the pathogenesis of human AAA and in targeting the development of new therapies for the management of AAA.

Aim: To determine the effects of calcium phosphate on gene expression in the infrarenal aorta.

Methods: Aortae from HFD fed Apoe−/− – mice compared to normal chow fed Apoe−/− – mice (n=3) were treated with 5 µM TNFα -stimulated HUVECs, was reduced by as by P<0.001, but not by GTN (P<0.05), whilst the cytokines MCP-1 and IL-6 were reduced by both as and GTN (P<0.05). In proof of concept experiments using mouse aortas stimulated with TNFα, both as (10 µM) and GTN (10 µM) attenuated TNFα-induced expression of leukocytes by 10 min (TNFα: 18 ± 2; TNFα + as: 4 ± 2; TNFα + GTN: 5 ± 1 leukocytes/field; n=10–15; P<0.001). The effects of as were abolished by the HNO scavenger, L-cysteine (3 mM; n=3; P<0.001), and ODQ (n=2; P<0.01) and GTN by hydroxocobalamin (n=3; P<0.001) but not ODQ. Expression of the transfection factor NFkB was increased with TNFα and both as and GTN reduced NFkB fluorescence intensity by 36% and 31%, respectively. The reduction in NFkB fluorescence by as was inhibited by ODQ (n=4–5; P<0.05).

Conclusions: These results show for the first time that HNO reduces inflammation, without the development of tolerance, through an endothelial sGC-dependent activation of NFkB, adhesion molecule expression and cytokine release. Therefore, HNO donors may be a viable therapeutic agent for the treatment of CVD.

H-069
INFLUENCE OF APOLIPROTEIN E, AGE AND AORTIC SITE ON CALCIUM PHOSPHATE INDUCED ABDOMINAL AORTIC ANEURYSM IN MICE

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Background: Abdominal aortic aneurysm (AAA) affects –5% men aged >65 years and is an important cause of morbidity and mortality. The study of AAA pathogenesis in humans is limited. The use of appropriate animal models could potentially have an important role in furthering the understanding of the pathogenesis of human AAA and in targeting the development of new therapies for the management of AAA.

Aim: To assess relevant features of AAA induced by calcium phosphate in a mouse model.

Methods: AAA was induced by perivascular application of calcium phosphate to the infra-renal aorta of 3 and 7 month old male mice. Infra-renal aortic diameter was assessed prior to AAA induction and 2 weeks later. AAA induction was assessed by calculating expansion of the infra-renal aortic diameter over 2 weeks. Blood pressure was measured by the tail cuff method, and plasma concentrations of total cholesterol, low density lipoprotein and very low density lipoprotein cholesterol, pro-inflammatory cytokines and matrix metalloproteinase-9 were measured using commercially available kits.

Results: The median expansion of the infra-renal aorta 2 weeks after AAA induction was significantly greater in mice that were deficient in apolipoprotein E than in the age- and gender-matched wild-type controls (276% versus 94.7%; P=0.02). Plasma low density lipoprotein/very low density lipoprotein cholesterol concentrations 2 weeks after AAA induction were positively correlated with the expansion of the infra-renal aorta induced by calcium phosphate (Spearman r=0.661; P=0.04). The median expansion of the infra-renal aorta 2 weeks after AAA induction was similar in 3 and 7 month-old wild-type mice (121% versus 126%; P=0.33). The local administration of calcium phosphate was associated with an increase in the mean maximal diameter of distant aortic segments, but not associated with changes in the concentrations of circulating pro-inflammatory markers.
THE CARDIOPROTECTANT 3',4'-DIHYDROXYFLAVONOL INHIBITS THE MITOCHONDRIAL PERMEABILITY TRANSITION PORE
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Background: 3',4'-Dihydroxyflavonol (DiOHF) reduces injury caused by myocardial ischemia and reperfusion (IR) in association with a reduction in oxidative stress. Oxidative stress contributes to the opening of the mitochondrial permeability transition pore (mPTP), a key event in myocardial IR injury.
Aim: To determine the effect of DiOHF on mPTP opening, mitochondrial respiration and the generation of reactive oxygen species (ROS) by cardiac mitochondria, after IR in anesthetised rats.
Methods: Male Wistar rats were anesthetised with pentobarbitone (70 mg/kg, i.v.) and ventilated. The left main coronary artery was occluded for 30 min and reperfused for 15 min. The heart was rapidly removed and the area at risk of ischemia was homogenized and centrifuged to isolate the mitochondria. Sham rats were anesthetized but not subjected to ischemia. DiOHF (10 mg/kg, i.v.) or vehicle (DMSO) was administered 5 min before reperfusion. mPTP opening was measured by mitochondrial Ca2⁺ retention capacity. Mitochondrial O2 consumption was measured in the presence of pyruvate (5 mM) and malate (5 mM) with a Clark electrode and ROS generation was measured as the rate of H₂O₂ production using Amplex red.
Results: Treatment of sham rats with DiOHF significantly increased the concentration of Ca2⁺ required to stimulate mPTP opening (IR 87±6; sham+DiOHF 120±9 μM). This was accompanied by an increase in state 3 O₂ consumption (sham 532±55; sham+DiOHF 572±21 nmol O₂/min/mg protein) and a decrease in H₂O₂ release (sham 0.028±0.002; sham+DiOHF 0.019±0.002 nmol/mg protein). IR significantly decreased the concentration of Ca2⁺ required to stimulate mPTP opening (IR 44±5 μM), decreased state 3 O₂ consumption (IR 332±15 nmol O₂/min/mg protein) and increased H₂O₂ release (IR 0.034±0.001 nmol/mg protein) compared to sham. Treatment with DiOHF prevented IR-induced changes in mPTP opening (IR+DiOHF 78±5 μM, state 3 O₂ consumption 375±30 nmol O₂/min/mg protein) and H₂O₂ release (IR 0.028±0.002 nmol/mg protein) so that there was no difference compared to sham.
Conclusion: In normal rats DiOHF inhibits mPTP opening and decreases mitochondrial ROS production. Importantly, DiOHF administration before reperfusion prevents IR-induced mPTP opening, impairment of state 3 respiration and increases in ROS production. The beneficial actions of DiOHF on mitochondria are likely to make a major contribution to its cardioprotective actions.

H-071 BILIRUBIN: A NOVEL ENDOGENOUS HYPOLIPIDEMIC AND HYPOTENSIVE AGENT PREVENTING CARDIOVASCULAR DISEASE
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Background: A clear relationship exists between circulating bilirubin and coronary atherosclerotic disease, with mildly elevated bilirubin being protective. Despite bilirubin's well-known antioxidant effects possibly contributing to atheroprotection, the effect of bilirubin on lipid status and systolic heart function remains unknown.
Aim: To determine whether elevated bilirubin is associated with hypotension and hypolipidemia in patients with benign hyperbilirubinemia.
Methods: Eight 0-80 mmol/l bilirubin controls undergoing coronary artery bypass graft surgery were randomized to continue with their standard diet (23) that was of comparable magnitude to that seen in the Lewis controls. Losartan administration did not affect baseline SNA in either strain, but reduced AP significantly in both Lewis and LPK (-36±3 and -16±4 mmHg, respectively). After losartan, the AP response to activation of peripheral chemoreflex was not altered in either strain, but were the SNA responses in the Lewis strain. Losartan normalized the sympathetic peripheral chemoreflex response in the LPK, however, confirming a significant role of the chemoreflex in modulating arterial blood pressure in both strains. Both strains demonstrated similar increases in baseline SNA and baseline SNA was not altered in either strain, but were the SNA responses in the Lewis strain.
Conclusion: These data indicate a role for the RAS in the altered sympathetic responses observed after activation of the peripheral chemoreflex observed in the LPK model of CKD.

H-072 THE AORTIC RESERVOIR IS A GENUINE PHYSIOLOGICAL PARADIGM: FIRST STUDY IN HUMANS
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Background: Central (aortic) blood pressure (BP) predicts mortality, but the physiological mechanisms underlying aortic BP waveform morphology are debated. The “aortic reservoir” is proposed as a component of aortic BP; but this relationship has only been assessed using a mathematically-derived-aortic reservoir-excess pressure model (AR_e). For the first time, the present study aimed to directly measure the aortic reservoir (AR_e) by cyclical change in aortic volume and determine the relationship with AR_e and aortic BP.
Methods: Ascending aortic BP and Doppler flow velocity were recorded via intra-arterial wire in 10 males (aged 62±2 12 years) during coronary artery bypass surgery. Simultaneous ascending aortic transesophageal echocardiography was used to measure AR_e. Published mathematical formulae were used to calculate AR_e.
Results: AR_e was strongly and linearly related with AR_e during systole (r=0.988; P<0.001) and diastole (r=0.985; P<0.001). Peak cross-correlation (r=0.98) occurred at a phase lag of 0.004 seconds into the cardiac cycle, suggesting close temporal agreement between waveforms. The relationship between aortic BP and AR_e was qualitatively similar to the cyclical relationship between aortic BP and AR_e with peak cross-correlations occurring at almost identical phase lags (AR_e vs. aortic BP; r=0.96 at 0.062 seconds and AR_e vs. aortic BP; r=0.98 at 0.057 seconds).
Conclusion: Mathematically-derived aortic reservoir pressure is highly correlated with changes in proximal aortic volume, consistent with its physiological interpretation as corresponding to the instantaneous volume of blood stored in the aorta. Thus, the aortic reservoir paradigm has a genuine physiological basis and should be considered when interpreting central BP waveform morphology.
to modulate gene expression and likely contribute to most, if not all, neurological processes. Changes in miRNA in brain tissue in response to stroke have not, however, been reported.

**Aim:** To investigate the functional role of brain miRNAs and gene regulatory networks in stroke injury.

**Methods:** Adult (8–12 weeks of age) male C57BL/6 mice underwent intravenous filament-induced middle cerebral artery (MCA) occlusion. Permanent ischemia (no reperfusion) or MCAO + 24 h reperfusion occurred for 24 h and 40 min, respectively. Sham surgery with reperfusion (S+R) was completed after 30 min of MCA followed by 23.5 h reperfusion. Sham-operated mice (n=8) were used as controls. Total RNA was isolated from mouse brains and gene arrays (Affymetrix) and miRNA arrays (TaqMan OpenArray microRNA) were carried out. Validation studies were performed using RT-PCR and TaqMan individual assays.

**Results:** InShi significantly altered (P<0.05; fold-change ≥1.5) the levels of 471 miRNAs in the brain, as compared to sham mice. By contrast, IR resulted in only 114 significant gene expression changes at 24 h. We found 7 miRNAs to be down-regulated and 1 miRNA to be up-regulated by reperfusion. Brain miRNAs were also very sensitive to both ischemia and reperfusion. We found 28 brain miRNAs (11 downregulated, 17 upregulated) to be significantly altered with either atrium or ventricle in different cardiac disease settings. The underlying dysregulation of this hypothesis is that with age this pathway may no longer operate in females.

**Aim:** To determine if the AT1R-mediated attenuated pressure response to Ang II is present in aged females.

**Methods:** Mean arterial pressure (MAP) was measured via telemetry in adult (20 week-old) and aged (65 week-old) FVB/N wild-type (WT) and AT1R knockout (KO) female mice during baseline and 14 day infusion of vehicle (saline) or Ang II (600 ng/kg/min). Renal expression of the ATâ€‘R and AT1R was determined using real time RT-PCR.

**Results:** Basal MAP was similar between the adult females (WT 93 ± 1 mmHg; n=13; AT1R-KO 93 ± 1 mmHg; n=12). With age, there was no change in basal MAP (aged WT 93 ± 1 mmHg; n=11; aged AT1R-KO 93 ± 1 mmHg; n=14). In the 20-week old adult females, the pressure response to Ang II was significantly attenuated in the WT as compared to the AT1R-KO (29 ± 3 mmHg vs 10 ± 4 mmHg, respectively, on day 14; P<0.01). The pressure response to Ang II was, however, augmented in the aged WT as compared to the 20-week old adult WT mice (P<0.01). Consequently, the increase in MAP in response to Ang II was similar between aged WT and AT1R-KO females (34 ± 3 mmHg vs 31 ± 4 mmHg, respectively, on day 14; P>0.05). In WT females, aging was associated with an increase in the renal AT1R/AT1R ratio.

**Conclusion:** The augmented pressure response to Ang II in the aged female WT mice demonstrates that the protective role of the AT1R depressor pathway is lost with age. Loss of this mechanism may contribute to the sharp rise in arterial pressure post menopause. Consequently, targeting deficits in AT1R expression and/or signaling represents a novel therapeutic approach for postmenopausal hypertension.
Methods: Embryos were either transferred to an intermediate ewe (ET) or cultured in vitro in the presence of human serum for 24 hours. Some of the embryos were cultured in the presence of a methyl donor (5-AzC) for 6 days. Naturally mated (NM) ewes acted as controls. At 24 weeks after birth, hearts were weighed, extracted and mRNA and protein expression of molecules involved in IGF-PI3K pathway were measured.

Results: There was no difference in the heart weight relative to body weight in any of the treatment groups compared to NM. There was no difference in the expression of IFG-1 mRNA and IFG8 mRNA in any of the treatment groups compared to the NM group. There was no difference in the abundance of protein kinases B (PKB/AKT) or its phosphorylated form, mammalian target of rapamycin (mTOR) and phosphorylated mTOR, as well as S6 and its phosphorylated form between the treatment groups.

Conclusion: This study suggests that the increase in heart weight in fetal life that is associated with embryo transfer or in vitro embryo culture do not persist in postnatal life. In addition, embryo transfer and in vitro embryo culture do not result in a activation of the IGF/PI3K (P110α) signaling pathway in the heart in postnatal life.

H-103 IMPACT OF IN VITRO CULTURE AND EMBRYO TRANSFER ON CARDIAC CONTRACTILITY IN THE HEART OF SINGLETON AND TWIN SHEEP FETUSES

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Background: Studies have shown that assisted reproductive technologies are associated with abnormal cardiac development.

Aim: To investigate the effect of in vitro culture and transfer of the embryo on signaling molecules involved in cardiac contractility in singleton and twin sheep fetuses.

Methods: Embryos were cultured in HEPES buffered saline for 72 hours, and were either transferred to an intermediate ewe or cultured in vitro in the absence (IVC group; singletons=5, twins=7) or presence of human serum (IVCH group; singletons=6, twins=4) for 6 days before transfer to recipient ewes. Naturally mated (NM) ewes were used as controls (singletons=4, twins=8). At 144/145 days gestation, ewes were killed and hearts were dissected and tissues were snap frozen in liquid nitrogen. Protein abundance was measured by Western blotting.

Results: There was no change in abundance of protein kinase C-α (PKC-α) or troponin I in all treatment groups compared to controls in both singletons and twins. The protein abundance of phospho-PhosHC and phospho-troponin I did not change in any treatment group in singleton fetuses. There was, however, a decrease in the protein abundance phospho-PhosHC (P<0.05) and phospho-troponin I (P<0.001) in the IVC and IVCH groups, in twins only. Culture and transfer of the embryo did not alter the protein abundance of SERCA in any treatment group in either singletons or twins.

Conclusion: The present findings suggest that in vitro embryo culture and transfer may affect the contractility of cardiomyocytes in twins, but not singletons. If a decrease in contractility persists, the individual may be at an increased risk of cardiovascular disease in later life.

H-104 WIDESPREAD CORONARY ENDOTHELIAL DYSFUNCTION IN THE ABSENCE OF HDL RECEPTOR SR1B IN A MOUSE MODEL OF ISCHEMIC CARDIOMYOPATHY

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Background: Occlusive coronary artery disease is a leading cause of morbidity and mortality. While extensive characterization of lipoproteins by HDL starting receptor class B1 (SR1B) plays an important role in the progression of atherosclerosis, it is unclear how SR1B influences coronary endothelial function in the face of high levels of oxidized LDLs. Here we utilized mice with hypomorphic ApoE lipoprotein (hyp) that lacked SR1B (* or were heterozygous for SRIB (+)) to investigate coronary control in atherogenic state.

Aim: To determine the extent of endothelial dysfunction in vivo in the coronary circulation of male mice after exposure to a Paigen high fat diet for 7 days and normal chow for a further 2 weeks.

Methods: 8 hyp−/− and 10 hyp+/+ mice were compared with synchrotron microangiography cine recordings during baseline, acetylcholine (ACh), sodium nitroprusside (SNP) infusions and vehicle or ACh/ACh infusions after NOS and COX inhibition with L-NAME (50 mg/kg) and sodium meclofenamate (3 mg/kg).

Results: Significant endothelium-dependent or independent dilator responses were absent in hyp−/− mice across the 1st to 4th branching order arterial segments (ACh and SNP). Partially occlusive stenoses were clearly observed in angiograms. Vessel segment adjacent stenoses displayed constriction following NOS/COX blockade, while globally non-stenotic segment did not alter significantly from baseline. Although hyp−/− mice did not show dilatation of vessel caliber there were significant increases in visualized vessel number during infusions of ACh, SNP and ACh post blockade, in both small and large vessels (P<0.01–0.05).

Conclusion: A high fat diet induced widespread coronary endothelial dysfunction ahead of occlusive human atherosclerosis. The absence of caliber changes suggest that SRIB is essential for nitric oxide-mediated coronary dilation. Interestingly, in the absence of SRIB the role of EDHF as a coronary dilator was variable between animals, but important for the maintenance of coronary flow through the opening of medium to small epicardial and penetrating transmural arteries and arterioles in mice after exposure to the Paigen diet. Enhanced EDHF production is likely to be important for the maintenance of larger vessel calibres in mice lacking SRIB or when SRIB expression is downregulated by oxidative stress.

H-105 CHANGES IN MICRO-RNA EXPRESSION IN THE AGING HUMAN HEART

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Background: Aging is associated with disproportionate prevalence of cardiovascular diseases, even in cohorts with low cardiovascular risk. The anatomic, functional and molecular changes that occur in the heart over the course of aging render this organ more vulnerable to various stressors, favoring the development of cardiac disease. MicroRNAs (miRNAs), a class of small non-coding RNA molecules of ~22-23 nucleotides in length, regulate the expression of protein-coding genes by post-transcriptional mechanisms and play a role in the cardiac remodeling that occurs during the postnatal period and in aging. Importantly, the profile of cardiac miRNA expression was shown to change during aging in animal models. A systematic study combining the analysis of global miRNA expression in aging human hearts with functional assessment of their role in cardiomyocyte sensitivity to stress has not yet to be conducted.

Aim: To determine the role of miRNAs in regulation of survival and cell death signaling pathways in the aging human heart.

Methods: We used miRNA 3.0 arrays (Affymetrix) and Partek Genomic Suite for data analysis to compare the expression profiles of miRNAs isolated from hearts of human fetuses (age 20–31 weeks; n=3), young adults (24–32 years old; n=3) and elderly adults (68–76 years old; n=3).

Results: The comparison of fetal vs. young and elderly groups identified 6 miRNAs with significantly altered expression (FDR<0.05, and fold change threshold of ≥1.5). Of these, 3 miRNAs (mir-108b-3p, mir-22b-2 and mir-664) were also differentially expressed between the young and the elderly groups. These miRNAs have been linked to regulation of autophagy and fibrosis, and associated with aging and/or atrial fibrillation in animal models.

Conclusion: Aging is associated with changes in the expression of human cardiac miRNAs. In future studies we aim to confirm expression profiles of these miRNAs in an extended sample set, and conduct functional studies in human cardiomyocytes derived from induced pluripotent stem cells.

H-106 ROLE OF MICRO-RNA IN CARDIOPROTECTION BY A RENIN-ANGIOTENSIN SYSTEM INHIBITOR IN AN ANIMAL MODEL OF RENAL INJURY

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Background: Cardiac hypertrophy and structural abnormality are key pathologies that contribute to the high rate of cardiac death in humans with kidney disease. Elevated renin-angiotensin system (RAS) activity plays an important role in the pathophysiology of heart complications in kidney disease. Thus RAS inhibitors are commonly used to minimize heart pathology in these patients. MicroRNAs (miRs) are small endogenously transcribed regulatory RNAs. They modulate gene expression by binding to 3′ or 5′ untranslated regions (UTR) of miRNAs, and have been shown to act as circulating biomarkers of heart damage following myocardial infarction. Animal studies have reported that dysregulated levels of mir-1, mir-133b, mir-133a, mir-208a, mir-208b, mir-499 and mir-21 in heart tissue collected from an animal model of renal injury. To investigate expression of miR-1, miR-133b, miR-133a, miR-208a, miR-208b, miR-499 and miR-21 in human tissue collected from an animal model of renal injury.

Method: Heart tissues collected from rats 10 days after subtotal nephrectomy (STNx), when the kidney was remixed and the other partially ligated (n=7), was compared with sham (n=7) rats or STNx rats (n=7) treated with the ACE inhibitor ramipril. RNA was extracted from hearts collected from the rats and real-time quantitative PCR (qPCR) was used to measure miR expression levels.

Results: We found that mir-208a, mir-208b and mir-21 were significantly (P<0.05) up-regulated in heart of STNx rats. There was a trend towards mir-1 up-regulation, but this did not reach statistical significance (1.5 fold increase; P=0.09). There was no change in mir-133b levels in hearts collected from STNx compared to the respective sham rats. Compared to vehicle-treated rats, treatment of STNx rats with ramipril did not prevent the observed increase in levels of mir-208a, mir-1, mir-208b and mir-21 in heart, but significantly increased the expression of mir-499 and mir-133a (P<0.01).

Conclusion: The cardioprotective action of ACE inhibition in an animal model of acute kidney injury could be mediated, at least in part, by up-regulation of mir-499 and mir-133a expression.
H-107 DEVELOPMENT OF A CARDIOVASCULAR MORTALITY RISK PREDICTION MODEL FOR PARTICIPANTS IN THE OHASAHA STUDY

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Background: Ambulatory blood pressure monitoring provides a more accurate blood pressure (BP) assessment than isolated clinic measurements, but can be more intensive and expensive than clinic measures. The extent to which novel BP indices from ambulatory monitoring improve cardiovascular risk prediction is less clear.

Aim: To develop a cardiovascular mortality risk prediction model for participants in the Ohasaha Study in Japan, examining conventional and novel indices of ambulatory blood pressure.

Methods: The study used 16.8 years of follow-up data from 1,535 participants in the Ohasama study. Our risk model was developed using univariate and multivariable Cox proportional hazards models. Variable selection was done using bootstrap methods on 1000 bootstrap samples. After development the model was validated using a 500 fold bootstrap validation method.

Results: Ambulatory and conventional systolic BP were obtained from 1,535 participants with average age of 61.7 ± 10.750 years, of whom 63% were women. In this population, 8.2% experienced a fatal cardiovascular event. The final cardiovascular risk prediction model included risk factors such as age, smoking status, number of antihypertensive medications, mean daytime ambulatory systolic arterial blood pressure and difference between daytime and nighttime ambulatory systolic arterial blood pressure (C statistic was 0.822). The ambulatory variables predicted cardiovascular mortality better than conventional BP variables. The internal bootstrap validation provided us with a C statistic of 0.817.

Conclusion: This risk prediction model incorporating novel indices from ABPM provides a direct risk assessment of cardiovascular mortality in Ohasama study participants, it could be externally validated and compared with other relevant cardiovascular mortality risk prediction models.

H-108 THE IMPACT OF AN EDUCATION PROGRAM TO REDUCE THERAPEUTIC INERTIA IN PRIMARY CARE MANAGEMENT OF HYPERTENSION

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Background: Therapeutic inertia is a term used to describe the situation in which there is reluctance to modify/intensify a treatment regimen when target treatment goals remain unmet. Therapeutic inertia has been identified as a significant contributor to the burden of uncontrolled blood pressure, and it has been suggested that uncertainty surrounding measurement and interpretation of blood pressures by primary care physicians may be a major factor.

Aim: To determine whether delivery of an education intervention focussing upon guideline-driven hypertension management to Australian General Practitioners (GPs) could reduce therapeutic inertia.

Methods: GPs enrolling in a clinical audit program were randomized to either control or therapeutic inertia program focussing on the challenges of, and strategies for, blood pressure management, with five of the six modules being interactive face-to-face workshops and one being a self-driven learning activity.

Results: Both the control and education groups demonstrated improvements in therapeutic inertia score from the first to second audit.

Conclusion: The results of this study suggest that participation in an audit of hypertension management was as effective as an intensive face-to-face program of education in addressing therapeutic inertia in general practice.

H-109 ENDOTHELIAL MINERALOCORTICOID RECEPTORS REGULATE DEOXYCORTICOSTERONE/SALT-MEDIATED CARDIO REMODELING AND VASCULAR REACTIVITY, BUT NOT BLOOD PRESSURE

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Background: Recent studies have described cell-specific roles for mineralocorticoid receptor (MR)-signaling in the cardiovascular system in terms of blood pressure regulation, recruiting and activation of immune cells, and promotion of cardiac tissue remodeling.

Aim: Firstly, to identify the role of endothelial cell MR in the vascular function of vehicle and aldosterone-treated mice, and secondly, to define the contribution of endothelial cell MR to doxycorticosterone (DOC)/salt-mediated inflammation and cardiac damage.

Methods: The vascular function of wild-type (WT) and endothelial cell MR-null (EC-MRKO) mice treated with vehicle or aldosterone (0.72 mg/kg/day) for 2 weeks was assessed ex vivo.

Results: Endothelial nitric oxide function was impaired in the thoracic aorta and mesenteric arteries of aldosterone-treated WT mice. While endothelial nitric oxide function was equivalently impaired in the mesenteric arteries of aldosterone-treated EC-MRKO mice, endothelial function was unaffected in the aorta, suggesting a differential role for endothelial cell MR depending on the vascular bed. At 8 days, loss of endothelial cell MR prevented DOC/salt-induced macrophage infiltration and the increased expression of proinflammatory genes in the myocardium. Cardiac collagen content was equivalent between genotypes at 8 days, although mRNA levels of profibrotic genes were significantly lower in EC-MRKO mice versus WT mice. At 8 weeks DOC/salt treatment increased macrophage recruitment and proinflammatory gene expression in WT mice but not EC-MRKO mice. Cardiac collagen deposition and CTGF mRNA levels were significantly reduced in EC-MRKO mice versus WT mice. Interestingly, systolic blood pressure was equivalently elevated in DOC/salt-treated WT and EC-MRKO mice at 8 weeks.

Conclusion: Our data demonstrate that endothelial cell MR signaling contributes to vascular nitric oxide function in large conduit arteries but not resistance vessels. We have, moreover, highlighted an important and independent role for endothelial cell MR signaling in the cardiovascular proinflammatory and profibrotic response to DOC/salt.

H-110 PATIENTS WITH AT LEAST THIRTY PERCENT OF HOME SYSTOLIC BLOOD PRESSURE ELEVATIONS ARE LIKELY TO HAVE UNCONTROLLED BLOOD PRESSURE: A PRAGMATIC METHOD FOR DOCTORS TO ASSESS BLOOD PRESSURE CONTROL FROM PATIENT BLOOD PRESSURE DiARies

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Background. Home blood pressure (HBP) is a method that is superior to clinic BP for the measurement of usual BP. A pragmatic barrier to using HBP is the requirement of doctors to calculate mean BPs from patient diaries.

Aims. To develop a timely and pragmatic method for determining the optimal ratio of HBP readings above target (systolic BP ≥135 mmHg) that would best predict elevated ambulatory systolic BP.

Methods. HBP (≥2 morning and evening diaries readings over 7 days), 24-hour ambulatory BP and left ventricular mass index (LVM) using 3D-echoangiography were measured in 286 patients with uncomplicated treated hypertension (aged 64±8 years; 53% female). Uncontrolled BP was defined as 24-hour SBP ≥130 mmHg.Indices of model calibration (deviance) and classification (area under the curve, AUC; net reclassification index and integrated discrimination improvement) were used to determine the optimal number of systolic HBP readings (≥135 mmHg from the last 10 recorded) to predict those with uncontrolled BP.

Results. Having ≥3 of the last 10 systolic HBP readings ≥135 mmHg provided the best prediction of systolic BP above target/treatment threshold (AUC=0.71, 80% specificity and 62% sensitivity). These individuals also had LVM that was 1.28 g/m² (95% CI 0.05–2.61) higher than those that did not meet this criterion.

Conclusions. To facilitate uptake of HBP monitoring we propose that doctors can determine the percentage of the last 10 systolic HBP values above 135 mmHg and manage their patient accordingly.

H-111 PREVALENCE OF KHL3 AND CUL3 MUTATIONS IN FAMILIAL HYPERKALEMIC HYPERTENSION


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Background: Familial hyperkalemic hypertension (FHHt, Gordon’s syndrome) is an inherited form of salt dependent hypertension caused by mutations in genes encoding proteins sensitive sodium transporter) are present in our FHHt pedigrees previously screened and found negative for WNK1 and WNK4. More recently mutations in two more genes, Keilch-3 like 3 (KHL3) and Culin 3 (CUL3), have been implicated in the causation of this condition.

Aim: To examine whether mutations in KHL3, CUL3 or SLC4A4 (an alternative thiazide sensitive sodium transporter) are present in our FHHt pedigrees previously screened and found negative for WNK1 and WNK4 mutations.

Methods: 25 affected individuals from 16 families with unexplained FHHt underwent genetic analysis by next generation sequencing. Validation of results was by Sanger sequencing.

Results: Affected individuals from 10 of 16 families were found to have CUL3 or KHL3 variants not reported in the general population. Eight pedigrees carried variants previously associated with FHHt, two in CUL3 (change: c.1377+10-C; c.1207-10-A) and four in KHL3 (c.1499G–T; c.11607C–T in three pedigrees, c.1019C–T; c.1480G–A: two pedigrees) had previously unreported variants in CUL3 (c.1377+10-T; c.1207-12T-A).
and one individual was homozygous for a previously reported heterozygous KHLR3 variant (c.1409G>A). We found no evidence for disease causing variants in SLC4A8.

Conclusion: Overall, 63% of our WMK1 and WMK4 mutation-negative pedigrees now have a genetic diagnosis, implying mutations in other, as yet unknown, regulators of the NCC are likely to exist.

H-112 CHRONIC MID-LATE GESTATIONAL HYPOXIA LEADS TO HYPERTENSION IN MALE AND FEMALE MOUSE OFFSPRING

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Background: Fetal hypoxia is a common gestational insult characterized by the redistribution of cardiac output to favor the brain and heart. This occurs at the expense of peripheral organ development and is usually related to growth restriction. We hypothesized that these hypoxia-induced alterations to the fetal cardiovascular system may persist into adulthood and increase the risk of hypertension and cardiovascular disease.

Aim: To examine whether mid to late gestational hypoxia can increase the risk of hypertension and cardiovascular disease in aged mouse offspring.

Methods: Pregnant CD-1 mice were placed in a hypoxic chamber (12.9%, O2; 11%) or control (21% O2; n=11) environment from embryonic day (E) 14.5 to birth (E19.5). Systolic blood pressure (SBP) and diastolic blood pressure (DBP) was assessed in the offspring (control: male n=6, female n=5; hypoxia: male n=6, female n=6) at 14 months (mo) of age via radiotelemetry reading over three days. Mean arterial pressure (MAP), pulse pressure (PP) and heart rate (HR) were calculated from these parameters.

Results: Offspring exposed to maternal hypoxia were growth-restricted (P<0.01) at postnatal day 1. No differences in body weights were observed at 14 mo. Male and female offspring exposed to maternal hypoxia had significantly higher MAP throughout both the light and dark cycle compared to control counterparts (males: P=0.01, females: P=0.02). In male offspring, this was driven by an increase in DBP (P<0.0001) with no change in SBP. Maternal hypoxia-exposed male offspring also had lower PP compared to control (P=0.02). In contrast, female offspring had an increase in both SBP (P=0.0001) and DBP (P<0.0001), with no change in PP. No change in HR was observed in groups in male or female offspring.

Conclusion: Fetal hypoxia led to growth restriction, but catch-up growth was observed later in life. Both male and female offspring developed hypertension, but in male offspring this was associated with a lower pulse pressure. This may reflect decreased cardiac function in male offspring, and potentially an increased risk of cardiovascular disease and mortality. Examination of vessel function and structure is currently underway to determine whether changes can be attributed to adulthood hypertension.

H-113 THE FUNCTION OF ErbB4 RECEPTOR IN THE POSTNATAL MOUSE HEART

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Background: ErbB receptors are a subfamily of tyrosine kinase receptors that regulate cell proliferation and differentiation. Of the four subtypes of ErbB receptor (ErbB1–ErbB4), ErbB4 is the most abundant subtype in the postnatal heart. Upon activation by growth factors such as neuregulin 1 (NRG1), ErbB4 forms homodimers or heterodimers to initiate downstream activity of hypertrophic genes was measured using luciferase reporter assays, ERK1/2 activation was measured using western blot and cardiomyocyte hypertrophy was determined by an image analysis software using the MyoHelix promoter was used to selectively delete the ErbB4 receptor from cardiomyocytes in adult mice. ErbB4 deletion was confirmed using qPCR, and echocardiography was used to evaluate the expression of eNOS, VACM and ERK1/2 expression.

Aim: To examine whether ErbB4 deletion also affects cardiac hypertrophy, apoptosis and fibrosis.

Methods: Using Cre/Lox recombination we have successfully established a model of cardiac ErbB4 knockouts (25.5±2.6 vs 31.8±3.4% in non-flxed controls; n=5–8). This was not, however, statistically significant.

Conclusion: Activation of the ErbB4 receptor is required for NRG1-induced cardiomyocyte hypertrophy. Using Cre/Lox recombination we have successfully established a model of cardiac ErbB4 knockouts. Deletion of ErbB4 in adult mice tends to impair cardiac function. We are now examining whether ErbB4 deletion also affects cardiac hypertrophy, apoptosis and fibrosis.

H-114 NITRIC OXIDE (NO) REVERSED TNF-α INHIBITION OF TROPHOBLAST INTEGRATION INTO ENDOTHELIAL CELLULAR NETWORKS

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Background: The interaction between trophoblast cell and maternal uterine endothelium is important for placental vascular modeling. Nitrergic oxide (NO) is a potent vasorelaxant that regulates systemic blood pressure. Reduced NO has been seen in preeclampsia.

Aim: To examine whether NO has a role in regulating TNF-α-induced inhibition of trophoblast cell integration into endothelial cellular networks in vitro.

Methods: Red fluorescence-labeled human uterine myometrial microvascular endothelial cells (UMVECs) were seeded on Matrigel. After endothelial cellular networks appeared, green fluorescent-labeled HTR-8/SVneo trophoblast cells were co-cultured with endothelial cells, together with or without TNF-α (0.5 nmol/l) and/or the NO donor, sodium nitroprusside (SNP) (10−6 mol/l). The number of SNV cells integration were quantified by image analysis software (Image J). The cells were then recovered from Matrigel to extract mRNA. Quantitative PCR was performed to evaluate the expression of eNOS, VACM and ERK1/2 expression. The concentration of VACM and ERK1/2 in the conditioned medium was also seen. NO reversed the inhibitory effect of TNF-α on trophoblast integration and increased eNOS mRNA expression. NO also reduced the expression of ERK1/2 and VACM that were increased by TNF-α.

Conclusion: Our data suggest that the inhibitory effect of TNF-α on trophoblast integration may be mediated by NO, via a reduction in endothelial cell activation.

H-115 BLOOD-BRAIN BARRIER DISRUPTION FACILITATES INTRA-CARTILAGOUS ACTIVATION BY ANGIOTENSIN II OF TYROSINE HYDROXYLASE POSITIVE NEURONS IN THE RAT ROstral VENTROLATERAL MEDulla

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Background: Angiotensin II (Ang II) plays an important role in blood pressure control in both the periphery and brain. With the exception of the circumventricular organs, circulating angiotensin is excluded from the brain by the blood-brain-barrier (BBB).

Aim: To investigate whether BBB disruption leads to increased activation by circulating Ang II of tyrosine hydroxylase (TH)-containing neurons in the rostral ventrolateral medulla (RVLM).

Methods: Male SD rats were anaesthetized (Pentobarbitone, 60 mg/kg, i.p.), prior to BBB disruption with intracardiac infusion of mannitol (1.6M, 2 ml/kg) followed by subsequent intracardiac infusion of a subpressor dose of Ang II (5ng/kg). Rats were then perfused and the brains were processed for TH and Fox immunohistochemistry.

Results: Ang II activated ~24% of TH-containing RVLM neurons, whereas only ~8% of TH cells were activated in the saline group. Intracardiac pretreatment with the Ang II receptor type 1 (AT1) blocker, losartan (20 μg/kg), significantly reduced the number of TH cells activated to ~11% (P<0.05 by one-way ANOVA; n=5).

Conclusion: Disruption of the BBB resulted in increased activation by circulating Ang II of TH-containing cells in the RVLM. This was blocked by losartan, indicating a specific action on AT1 receptors. These results suggest that disruption of the BBB allows entry of circulating Ang II into the RVLM, which might act to increase sympathetic outflow.

H-116 DOWN-REGULATED Ca2+/CALCUMODULIN-DEPENDENT KINASE II ACTIONS IN THE HYPTERTOPIC FEMALE HEART – A LIABILITY FOR ISCHEMIA/REFPERUSION ARRHYTHMIAS?

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Background: Women are less susceptible to ischemia-related lethal arrhythmias than men, yet female (but not male) risk increases substantially with an underlying hypertrophic pathology. With a continuing rise in obesity, diabetes and concomitant hypertension, the impact of hypertension on ischemia-related arrhythmias/mortality in women would be expected to increase. Ca2+/calmodulin-dependent kinase II (CaMKII) is a key regulator of myocardial Ca2+-handling proteins, which mediates cardiac hypertrophy, failure, and atrial/ventricular arrhythmias, yet its role in generating reperfusion arrhythmias in hypertrophic hearts has not been investigated.

Aim: To determine the mechanistic relationship in female cardiac hypertrophy linking CaMKII, phospholamban phosphorylation at the CaMKII-specific residue (PLb-Thr17) and arrhythmias, using the female hypertrophic heart rat (HHR) model of primary cardiac hypertrophy.
H-117

NEURONAL ACTIVATION IN THE HYPOTHALAMUS, MIDBRAIN AND MEDULLA FOLLOWING MYOCARDIAL INFARCTION IS INHIBITED BY MINOCYCLINE

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Background: Following myocardial infarction, neuronal activity is elevated in the hypothalamic paraventricular nucleus (PVN) suggesting this nucleus contributes to the autonomic reflex responses observed in ventricular dysfunction. There is little is known about effects on other brain nuclei.

Aims: To investigate (i) whether the rostral ventrolateral medulla (RVLm), the nucleus tractus solitaries (NTS) and the periaqueductal gray (PAG), regions known to have important cardiovascular regulatory functions, also show increased neuronal activation; (ii) the effect of administering the anti-inflammatory drug, minocycline into the brain ventricles on neuronal activation and heart function.

Methods: Sprague Dawley rats were infused with either saline (0.05% NaCl or minocycline (172 mg/ml, 0.3 μl/h) into the lateral ventricle of the brain. The rats then underwent either a myocardial infarction (MI) or sham procedure. Cardiac function was determined by echocardiography 12 weeks post MI. The rats were then killed and the brains processed for immunohistochemistry to detect changes in neuronal activity using the presence of the marker proteins, Fos related antigens (FRA).

Results: MI elicited a significant increase in FRA in the PVN, RVLm, NTS and PAG (P<0.001; n=3). Minocycline significantly attenuated the responses by at least 50% in the PVN and almost completely in the PAG, RVLm and NTS (n=5). Cardiac function was significantly reduced by 55% following MI, but this was not ameliorated by minocycline.

Conclusion: Following MI there is increased neuronal activity in brain nuclei that play key roles in cardiovascular regulation. Attenuation of this response may not be sufficient to improve cardiac function.

H-118

SELECTIVE CARDIOVASCULAR ADAPTATION TO CHRONIC STRESS IN MICE

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Background: The cardiovascular effects of acute emotional stress are well known and include increases in blood pressure (BP) and heart rate (HR). There is habitatuation with chronic stress leading to less cardiovascular effects. However, the cardiovascular effects of a novel stress may be similar or possibly enhanced.

Aim: To develop a model of chronic stress in conscious mice in order to investigate BP as well as cardiovascular responses to repeated and novel stressors.

Methods: Male C57Bl/6 mice were implanted with telemetry probes and recordings of mean arterial pressure (MAP) and HR were made in non-stressed (n=4) and chronically stressed mice (n=10; 2 hours pressure per day for 3 weeks). The daily stress included a random combination of 60 min of restraint and 2×30 min of placement in a cage that had previously been occupied by another male mouse. Shaker stress was chosen as the novel stress and involved placing the mice in its home cage on a slowly rotating orbital platform for 5 min.

Results: Exposure to chronic stress had no effect on basal levels of BP or HR, but attenuated the pressor (14.0 vs 21.2 mmHg; P<0.01) and tachycardic (95 vs 219 bpm; P<0.001) responses to dirty cage switch. No differences were observed in the cardiovascular response to restraint stress. There was, however, a marked increase in the response to the novel shaker stress in that MAP increased by +7.1 mmHg (P<0.01) and HR increased by +70 bpm (P=0.05) compared with responses from non-stressed mice.

Conclusion: The findings shows that cardiovascular responses to chronic stressors do not always lead to habituation. Responses to novel stressors can be enhanced and adaptation to long-term stressful situations may be non-uniform and may depend on the type of stress involved. Furthermore, the non-uniform adaptation suggests that the modulation likely involves higher brain regions such as the amygdala, rather than lower common autonomic pre-sympathetic pathways.
90 days gestation. ACE1 gene expression was lower in IUGR fetuses at 140 days gestation. There was however a delay in the rise of ACE2 gene expression at 90 days gestation in IUGR fetuses. There was no effect of IUGR on cardiac expression of adrenergic receptor-β2 (ADRB1) mRNA, but interestingly adrenergic receptor-α2 (ADRA2) was higher at 55 days, 90 days and 140 days gestation in the IUGR group compared to controls.

Conclusions: These data show that NGF expression is higher in the heart of the IUGR fetus corresponding to sympathetic innervation of the sheep fetus. This may be related to the changes in local angiotensin II expression (to be measured) resulting in altered sympathetic innervation of the heart. These effects may be magnified by changes in cardiac expression of AR and underlie an increased vulnerability to cardiovascular disease.

# H-120
FUNCTIONAL REGROWTH OF SYMPATHETIC NERVES IS INCOMPLETE FOLLOWING LONG-TERM RENAL DENERVATION IN THE SPONTANEOUSLY HYPERTENSIVE RAT

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Background: Renal denervation (RDN) can attenuate the development, or delay the onset, of hypertension. Ongoing clinical trials using RDN have shown a sustained reduction in arterial pressure in humans with resistant hypertension. The mechanisms, however, contributing to this sustained decrease in arterial pressure remain unclear.

Aim: To examine mean arterial pressure (MAP) and functional sympathetic reinnervation of the renal vasculature in spontaneously hypertensive rats (SHR) following RDN.

Methods: 8 week-old male SHR were implanted with a radio telemetry probe to measure MAP. At 10 weeks of age and following a 6-day basal MAP recording, rats underwent surgical RDN (n=7) or a sham operation (n=6) and MAP was recorded until 22–26 weeks of age (12–16 weeks post-RDN). At the end of the recording period, segments of the renal lobar artery were isolated and mounted on a wire myograph and responses to perivascular nerve stimulation with single pulses were recorded simultaneously. Smooth muscle membrane potential was measured using intracellular microelectrodes. Thus membrane potential and tension were recorded simultaneously. Smooth muscle contraction to α1 - and α2-adrenoceptor agonists was also assessed.

Results: Basal MAP was not different between sham and RDN groups. MAP was significantly attenuated in RDN SHR, with the RDN group, being 16 ± 7 mmHg lower than the sham at 12 weeks post-RDN (P<0.04). Perivascular nerve stimulation with single pulses evoked excitatory junction potentials (eips). Eip amplitude was reduced in arteries from RDN SHR animals (P<0.01). Repetitive stimulation (1–8Hz; 5 eips) evoked slow depolarization and contraction. At 5 Hz or 5 s, slow depolarization amplitude was markedly smaller in RDN SHR arteries (12 ± 2 mV vs 8 ± 1 mV; P<0.05), with contractions being ~45% smaller in RDN arteries compared with sham (22 ± 3 % vs 12 ± 2 % of the contraction to the 100 mM potassium; P<0.03). There was no difference in smooth muscle sensitivity to phenylephrine, methoxamine or clonidine between the groups.

Conclusion: Our results suggest that there is a sustained decrease in MAP following RDN. The renal vasculature is reinnervated following RDN. The functional responses are not, however, restored even after 12–16 weeks. Smaller eips indicate that fewer neuromuscular junctions are reformed. Incomplete reinnervation of the renal vasculature may be a contributing factor to the sustained effect of RDN on blood pressure.

# H-121
WHOLE GENOME DNA SEQUENCING OF A GENETIC MODEL OF CARDIAC HYPERTROPHY IDENTIFIES TWO CANDIDATE GENES IN CARDIAC MASS QTL 22

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Background: Cardiac hypertrophy (CH) is a silent condition that involves thickening of heart muscle, so reducing functionality and increasing risk of cardiac disease and morbidity. Genetic factors are known to be involved, but their contribution is poorly understood.

Aim: To unveil genetic contribution to CH independent of blood pressure.

Methods: We sequenced the whole genome of the hypertrophic heart rat (HHR), a normotensive genetic model of CH, and its control, the normal heart rat (NHR) using the Illumina HiSeq 2000 platform. Clean reads were aligned and compared to the Rat Genome Database v3.4. We analysed four types of variants: single nucleotide polymorphisms (SNPs), copy number variations (CNVs), insertions and deletions (Indels) and structural variants (SVs).

Results: Overall, we found around 5.7 and 5.0 million variants in HHR and NHR, respectively. The majority of those variants (80%) were SNPs. Unique variants represented 27% and 16% of HHR and NHR mutations observed in all four types. HHR had around 4.5 million SNPs (25% unique), 100 thousand CNVs (all unique), one million Indels (29% unique) and 12 thousand SVs (89% unique), while NHR had almost 4 million SNPs (13% unique), 20 thousand CNVs (all unique), 960 thousand Indels (25% unique) and 11 thousand SVs (99% unique). In further analyses we identified variants in a locus we previously reported to control heart size independent of blood pressure on chromosome 2 (cardiac mass 22, former Lvm1) and correlated those with gene expression observed in whole-genome gene expression analyses in neonatal rats. Although two genes were differentially expressed, we only found mutations (19) in one of them. These mutations could contribute to the hypertrophic phenotype.

Conclusion: We have identified polymorphisms at the whole genome level in a genetic model of CH and pinpointed potential candidate genes for hypertrophy development located in the cardiac mass 22 QTL.

# H-122
INTRANERAL PERFUSION AND OXYGENATION IN AN OVINE MODEL OF SEVERE SEPSIS WITH HYPOTENSION AND KIDNEY INJURY

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Background: The pathophysiology of septic acute kidney injury (AKI) is poorly understood. It has been proposed that septic AKI may result from decreased perfusion and oxygenation of the renal medulla.

Aim: To establish the ability of chronically implanted optical fibre probes to monitor intrarenal perfusion and oxygenation in conscious sheep, and to determine the changes in perfusion and oxygenation in the renal cortex and medulla in an ovine model of severe sepsis.

Methods: Mean arterial pressure (MAP), cardiac output (CO), renal blood flow (RBF), and renal cortical and medullary tissue perfusion and oxygen partial pressure were measured in conscious sheep (n=8). Renal blood flow was reduced by 20% and 50% for 30 min with a vascular occluder on the renal artery. After 24 hours of baseline data collection, sepsis was induced with live E. coli into infusion for 24 hours.

Results: In the renal cortex and medulla, a 20% reduction in RBF decreased perfusion (14±6.8% and 41.2±8.5%, respectively) and oxygenation (48.1±8.5 and 72.4±8.5%, respectively). Following E. coli, all sheep developed a hyperdynamic state with a doubling in heart rate and CO, a 50% increase in RBF, and a 15 mmHg decrease in MAP. Urine output halved, serum creatinine doubled and creatinine clearance decreased by one third. Cortical perfusion and oxygenation did not change significantly, but medullary flow showed an early trend towards reduction, while medullary P50 progressively decreased by 53±11% at 24 hours of sepsis. Calculated total renal oxygen consumption did not change (33±32 ml O2/min).

Conclusions: Changes in RBF induced in this study predicted changes in intrarenal perfusion and oxygenation that returned to control levels after the occlusion was released, indicating responsiveness and stability of the measurements by the optical fibre probes. In a conscious ovine model of septic AKI, medullary oxygenation decreased, possibly due to reduced perfusion with unchanged oxygen consumption, or to intrarenal shunting. This reduction in medullary oxygenation may contribute to the development of septic AKI.

# H-123
IS THE HEART OF THE IUGR FETUS HYPOXIC IN EARLY GESTATION?

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Background: Intrauterine growth restriction (IUGR) and chronic hypoxemia cause a decrease in cardiomyocyte endowment in late gestation, in the absence of a change in the expression of hypoxia inducible factors (HIF1α, 1β and 2α) or genes with hypoxia response elements (HRE). We hypothesized that the decreased cardiomyocyte endowment in late gestation is due to either a decrease in proliferation or an increase in cardiomyocyte death in early gestation in the IUGR fetus.

Methods: A sheep model of IUGR was induced by removing endometrial caruncles (placental implantation sites). At 55 days gestation, control and IUGR ewes were killed humanely and hearts were weighed. The heart was weighed and ventricular samples were frozen and fixed. Gene expression was determined relative to the geometric mean of 3 housekeeping mRNAs using real-time PCR. The percent proliferating cardiomyocytes was determined with Ki67 staining. Protein expression was determined relative to a loading control using western blotting. Data were analysed using a Students’ t-test.

Results: The IUGR fetuses were smaller (control 0.33±0.001 kg; n=17; IUGR 0.22±0.001 kg; n=19), but there was no difference in relative heart weight. There was no difference in expression of HIF1α, 1β and 2α mRNAs or of genes with HRE including those for VEGF, IF2, IGF2R and GLUT1 in the IUGR group compared to controls. There was no change in the expression of mRNAs for proteins that promote cell cycle entry (Chek1, CDCA3, cyclinD1, cyclinD2 and O6mG), or in the percent of Ki67 positive cardiomyocytes or PCNA protein abundance between the IUGR and control fetuses. There was, however, a decrease in the protein expression of Bcl-x, an anti-apoptotic protein, and an increase in the protein expression of LC3B, a marker of autophagy in IUGR fetuses.

Conclusions: These data suggest that the heart of the IUGR fetus may not be hypoxic and that there is no change in cardiomyocyte proliferation in early gestation, there may be an increase in cardiomyocyte apoptosis or autophagy of cardiomyocytes. A small decrease in cardiomyocyte number early in gestation may thus result in reduced cardiomyocyte endowment in the IUGR fetus in late gestation.

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A POTENTIAL ROLE FOR RHO KINASE IN EARLY LEFT VENTRICULAR DYSFUNCTION IN DIABETES


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Background: Patients with diabetes have a 2–5 fold increased incidence of heart failure. Using synchrotron x-ray diffraction in the in situ beating heart, we have shown that crossbridge (CB) dynamics are impaired in the left ventricle (LV) of rats with early diabetes. Rho kinase (ROCK) plays an important role in regulating the phosphorylation state of proteins myosin light chain (MLC-2) and myosin binding protein–C (MyBP-C), which are involved in controlling myosin head leverage and CB dynamics in the myocardium. Furthermore, increased ROCK activity results in LV contractile dysfunction in animal models of diabetes.

Aim: To examine if early diabetes is associated with altered phosphorylation of proteins involved in regulating CB dynamics in early diabetes.

Methods: Type 1 diabetes was induced in rats by streptozotocin (i56 mg/kg, i.p.). Control rats received citrate vehicle. One week later, diabetic rats were further randomized to receive fasudil (10 mg/kg/day) or saline for 2 weeks. Rats underwent cardiac catheterization to assess LV function or were subjected to x-ray diffraction experiments at the SPring-8 Synchrotron, Japan to assess cardiac CB dynamics in situ.

Results: In comparison to control, 3 weeks of diabetes in rats resulted in prolonged LV relaxation times (P<0.05) and impaired systolic function (P<0.05). Fasudil mildly improved systolic function in diabetic rats. A trend for a 20% reduction of MyBP-C phosphorylation in diabetic rats compared to controls was shown and fasudil partially prevented this reduction in diabetic rats. MLC-2 phosphorylation was increased in diabetic rats, but fasudil did not affect MLC-2 phosphorylation. Furthermore, myosin head extension was impaired at end diastole in diabetic rats and was improved with fasudil treatment in the subendocardial layer of the LV wall.

Conclusion: Our results suggest that ROCK contributes to impaired LV contractility and CB dynamics in early diabetes, possibly by altering phosphorylation of proteins involved in regulating myosin head extension.

B CELL SUBSETS AND PATHOGENESIS OF ATHEROSCLEROSIS

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Background: Atherosclerotic lesions that develop in the vessel wall of medium/large arteries are the result of complex interactions between accumulated LDL-cholesterol, endothelial and smooth muscle cells and cells of the innate and adaptive immune system. Multiple immune cells accumulate in atherosclerotic lesions including macrophages and dendritic cells, NK and NKT cells, CD4+ and CD8+ T cells and B cells. Early studies indicated that B cells are protective against atherosclerosis by producing low affinity IgM antibodies against oxidised LDL (low density lipoprotein). However, recent advances in B cell immunobiology indicate multiple subsets of B cells, suggesting a more complex role in the pathogenesis of atherosclerosis.

Aim: To reinvestigate the role of B cells in atherosclerosis using fat fed ApoE−/− mice

Methods and Results: Using an anti-C020 B cell depleting antibody, we demonstrated that B cell depletion decreases development and progression of murine atherosclerosis. Adoptive transfer approaches demonstrated that the B2 cell subtype was proatherogenic. By targeting the B2 cell survival factor BAFF we demonstrated their important role in atherosclerotic lesion inflammation and development/progression of atherosclerosis.

Unlike B2 B cells, B1a B cells produce natural IgM antibodies as well as interleukin-10. In contrast to B2 B cells, deletion of peritoneal and splenic B1a B cells aggravates atherosclerosis. These effects were accompanied by marked reductions in anti-oxidized LDL IgM antibodies, lesion apoptotic cell numbers and necrotic core development. Administration of anti-TIM (RMT1-10) mAb to hyperlipidemic ApoE−/− mice expanded the B1a B cell population including TIM-1+IgM+ and TIM-1+IgM+IL-10+ B1a B cell subsets and attenuates atherosclerosis.

Conclusion: B2 B cells are proatherogenic, whereas B1a B cells are atheroprotective.

THE ROLE OF HIGH-DENSITY LIPOPROTEINS IN POLARIZING HUMAN MACROPHAGE PHENOTYPES

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Background: Macrophages play a critical role in the development and progression of atherosclerosis. Macrophages can polarize into two subtypes with distinctive phenotypes; inflammatory (M1) and anti-inflammatory (M2). High-density lipoprotein (HDL) has many cardioprotective properties including potent anti-inflammatory effects, largely through the removal of cholesterol from cells. It is currently not known if this extends to influencing human macrophage phenotype.

Aims: To investigate the effect of HDL on polarizing human macrophages to distinct phenotypes.

Methods: Human blood monocyte-derived macrophages were polarized to either an M1-phenotype by incubation with lipopolysaccharide (LPS: 100 ng/mL) and interferon-γ (IFN-γ: 20 ng/mL) or interleukin-4 (IL-4: 20 ng/mL) to induce M2-phenotype. Polarization was performed in the presence or absence of human HDL (400 μg/mL) or apoA-I (280 μg/mL). We examined cell surface markers, phagocytosis and ROS using flow cytometry and mRNA expression using real-time PCR. Downstream signaling pathways were also assessed by western blot analysis.

Results: We discovered that HDL inhibited polarization to M1 as evidenced by a decrease in the expression of the M1 surface marker CD192. This was accompanied by a decreased expression of the M1 inflammatory genes TNFA, IL6 and MCP1. HDL also inhibited M1 inflammatory function, with reduced phagocytic capacity as well as hydrogen peroxide and superoxide production. Similarly, apoA-I was also able to suppress M1 polarization (CD192) and inflammatory mRNA expression. We next explored the signaling cascades involved and found a decrease in ERK1/2 phosphorylation in M1 macrophages treated with HDL, suggesting cholesterol efflux inhibits the MAPKs during macrophage polarization to the M1 phenotype. However, HDL did not affect macrophage polarization to M2 phenotype.

Conclusion: We provide evidence that HDL not only reduces macrophage polarization to the inflammatory M1 phenotype, but also inhibits phagocytosis and ROS production in these cells. These data provide a new dimension to our understanding of the protective role of HDL and suggest that HDL may act to resolve inflammation in the atherosclerotic lesion.
THE ROLE OF NATIVE LOW-DENSITY LIPOPROTEINS (nLDL) IN MACROPHAGE POLARIZATION FROM DIFFERENT MONOCYTE SUBSETS


Background: Phenotypic changes in monocytes and macrophages (MO) after the progression of atherosclerosis. Differences in the three monocyte subsets (classical: CD14++CD16-, intermediate: CD14+CD16+, and non-classical: CD14+CD16-) have been associated with cardiovascular disease outcome. Monocyte derived MO can polarize into either the classically activated (M1) phenotype or an alternatively activated (M2) phenotype, depending on their microenvironment. Extracellular lipids can influence the heterogeneity of monocytes and MO, yet the manner in which this occurs is unclear.

Aim: We aimed to determine if the different monocyte subsets have an innate ability to differentiate and polarize to M0 and whether plasma lipoproteins can influence the outcome.

Methods: Primary monocytes or monocyte subsets were differentiated into macrophages (MO) by macrophage-colony stimulating factor (M-CSF; 100 ng/mL) for 6 days in the absence and presence of nLDL (200 ng/mL). MO were treated with lipopolysaccharide (LPS; 100 ng/mL) and interferon-γ (IFN-γ; 20 ng/mL) or interleukin-4 (IL-4; 20 ng/mL) for 6–24 hours to induce polarization to a M1 and M2 phenotype, respectively.

Results: Treatment with nLDL reduced the M2 surface markers, CD206 (M2: 320 ± 66 vs M2+nLDL: 159 ± 19, P<0.05; n=6), and CD200R (M2: 167 ± 20 vs M2+nLDL: 79 ± 12, n=8; P<0.05) expression in M2 macrophages. As well, mRNA expression of the cytokines TNFα and IL-10 was blunted in M2 macrophages treated with nLDL (n=3–4; P<0.05). MO differentiated into M2 from classical and intermediate, but not non-classical, monocytes expressed increased TGFβ and IL-10 mRNA levels, which were reduced 1.5-fold in monocytes from classical macrophages only by nLDL treatment (n=4; P<0.05). Alternatively, nLDL does not affect the expression of the M1 surface marker, CD64 (n=5; P>0.05), but does increase TNFα and IL-6 gene expression in M1 macrophages by 25- and 95-fold, respectively (n=3–4; P<0.05). Classical, intermediate and non-classical monocytes that were polarized to the M1 phenotype all had increased gene expression of TNFα and IL-6 in nLDL (n=3–4; P<0.05) and nLDL only increased TNFα and IL-6 mRNA levels in classical and intermediate macrophages, respectively (n=3–4; P<0.05).

Conclusion: nLDL reduces M2 macrophage polarization and enhances gene expression of the inflammatory cytokines TNFα and IL-6. Furthermore, both M1 and M2 macrophages derived from the different monocyte subsets respond differently to polarization stimuli and the effects of nLDL are subset specific.

POLARIZATION OF MONOCYTE SUBSETS TO AN M1 PHENOTYPE IN ATHEROSCLEROSIS


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Background: Monocytes and macrophages are integral to atherosclerotic plaque development, progression and, most importantly, rupture. The presence of macrophages in general (CD68), or a polarization towards an inflammatory (M1) phenotype or an alternatively activated (M2) phenotype, depending on the type of lipid added to the endosome, can be evaluated by macrophage-colony stimulating factor (M-CSF; 100 ng/mL) or interleukin-4 (IL-4; 20 ng/mL) for 6–24 hours to induce polarization to a M1 and M2 phenotype, respectively.

Aim: To review the lessons learned from these studies and potential implications for the design of future studies.

Methods: Primary monocytes or monocyte subsets were differentiated into macrophages by macrophage-colony stimulating factor (M-CSF; 100 ng/mL) for 6 days in the absence and presence of nLDL (200 ng/mL). MO were treated with lipopolysaccharide (LPS; 100 ng/mL) and interferon-γ (IFN-γ; 20 ng/mL) or interleukin-4 (IL-4; 20 ng/mL) for 6–24 hours to induce polarization to a M1 and M2 phenotype, respectively.

Results: Treatment with nLDL reduced the M2 surface markers, CD206 (M2: 320 ± 66 vs M2+nLDL: 159 ± 19, P<0.05; n=6), and CD200R (M2: 167 ± 20 vs M2+nLDL: 79 ± 12, n=8; P<0.05) expression in M2 macrophages. As well, mRNA expression of the cytokines TNFα and IL-10 was blunted in M2 macrophages treated with nLDL (n=3–4; P<0.05). MO differentiated into M2 from classical and intermediate, but not non-classical, monocytes expressed increased TGFβ and IL-10 mRNA levels, which were reduced 1.5-fold in monocytes from classical macrophages only by nLDL treatment (n=4; P<0.05). Alternatively, nLDL does not affect the expression of the M1 surface marker, CD64 (n=5; P>0.05), but does increase TNFα and IL-6 gene expression in M1 macrophages by 25- and 95-fold, respectively (n=3–4; P<0.05). Classical, intermediate and non-classical monocytes that were polarized to the M1 phenotype all had increased gene expression of TNFα and IL-6 in nLDL (n=3–4; P<0.05) and nLDL only increased TNFα and IL-6 mRNA levels in classical and intermediate macrophages, respectively (n=3–4; P<0.05).

Conclusion: nLDL reduces M2 macrophage polarization and enhances gene expression of the inflammatory cytokines TNFα and IL-6. Furthermore, both M1 and M2 macrophages derived from the different monocyte subsets respond differently to polarization stimuli and the effects of nLDL are subset specific.
PCSK9 IN HETEROZYGOUS FH (HeFH) PATIENTS

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Background: PCSK9 inhibition has been demonstrated recently to be an effective therapeutic approach to lower LDL in HeFH patients. The aim of the present study was to determine whether or not PCSK9 is similarly effective at inhibiting the LDL receptor (LDLR) in a wide range of HeFH molecular defects.

Aim: To assess the risk associated with elevated PCSK9 levels is greater in heterozygous FH patients.

Methods: PCSK9 levels were measured by ELISA in samples from normolipidemic controls (n=35) and in FH patients carrying either a D200G (n=18), a G361V (n=9), a P664L (n=9), a Del197 (n=4), a Thr369 Met228 (n=4), a Thr369 Met228 (n=4), and a P664L (n=1) mutation on one of their LDLR alleles. These LDLR variants were cloned and transiently expressed in HEK293 cells. Skin fibroblasts were also obtained from our FH patients. Cells were grown with 20% serum or 0.5% serum and increasing doses of mevasatin (0–40 mg/mL) were added to the cells for 0 to 6 days.

Conclusion: This large pooled analysis of four phase 2 studies demonstrated marked and significant reductions in LDL-C and favorable changes in other pro-atherogenic parameters in 1.4% vs 0.9% of patients receiving AMG 145 vs placebo. Injection-site reactions were more frequent with AMG 145 vs placebo (57% vs 49%; Table 2). AEs were more frequent with AMG 145 vs placebo (57% vs 49%; Table 2). Within AMG 145 doses vs 0.1% (1.6%) to 0.5% (1.4%) for placebo (∼0.001 for all dose groups). Favorable changes were also observed in Apo B, Lp(a), triglycerides, HDL-C, and Apo A1. The highest doses, AMG 145 140 mg Q2W (n=123) and 420 mg Q4W (n=213) vs placebo Q2W (n=123) and Q4W (n=178), produced the greatest efficacy (Table 1). AEs were more frequent with Amg 145 vs placebo (57% vs 49%; Table 2).

PCSK9 IN HETEROZYGOUS FH (HeFH) PATIENTS

A-010

DIET QUALITY AND ARTERIAL COMPLIANCE IN PEOPLE WITH TYPE 1 AND TYPE 2 DIABETES

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Background: People with diabetes are at higher risk of cardiovascular disease (CVD). There is evidence that better diet quality may be protective against CVD.

Aim: To investigate the association between indices of dietary quality and pulse wave velocity (PWV) in people with type 1 and type 2 diabetes.

Methods: Participants (age 59±13 years) were 66 adults with type 1 (n=5) or type 2 (n=61) diabetes recruited from the community. Dietary intake was measured using the 74 item electronic version of the Dietary Questionnaire for Epidemiological Studies (version 2) Food Frequency Questionnaire. Diet quality was assessed according to the a priori defined Australian Recommended Food Score (ARFS) and Healthy Eating Index (HEI). A SphygmoCor® XCEL (Sydney, Australia) was used to measure carotid-femoral PWV.

Results: The mean ARFS and HEI scores were 31.1±7.6 (score out of 74) and 62.1±13.0 (score out of 100) respectively. Mean PWV was 9.6±2.0 m/s. No correlation was observed between the ARFS or HEI and PWV. However, when individual components of the HEI were considered, there was a significant inverse association between the dairy score and PWV (r=-0.460, P<0.05). Total dairy intake (grams/day) was inversely associated with PWV after adjustment for age (r=-0.35, P<0.05). The observed correlation was attributable to milk consumption (r=-0.282, P<0.05). Yogurt and cheese consumption were not significantly associated with PWV.

Conclusion: In this cohort of subjects with type 1 and type 2 diabetes daily consumption, particularly milk intake, was inversely associated with arterial compliance. No association was observed between indices of diet quality and PWV.

A-013

FENUGREEK EXTRACT IS MORE EFFECTIVE THAN ITS ACTIVE COMPOUND, SAPONINS, IN ATTENUATING ENDOTHELIAL ACTIVATION IN HUMAN ARTERIAL ENDOTHELIAL CELLS

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¹The Centre for Pathology Diagnostic and Research Laboratories (CPDRL) and Institute of Medical Molecular Biotechnology (IMMB), Faculty of Medicine, Universiti Teknologi MARA, Sungai Buloh, Malaysia. ²Background: Inflammation and endothelial dysfunction are key events in the pathogenesis of atherosclerosis. There have been recent advances in identifying cardioprotective properties in natural products. Trigonella foenum graecum, more commonly known as fenugreek, is one compound that has attracted interest. However, to date, there have been few studies on the potential atheroscleroprotective properties of fenugreek and its active class of compounds, saponins.

Aim: To determine the effects of fenugreek crude extract and its saponins on protein and gene expression of endothelial activation biomarkers in stimulated human coronary artery endothelial cells (HCAECs).

A-011

THE EFFECT OF A HIGH POTASSIUM DIET ON VASCULAR FUNCTION

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⁴School of Pharmacy and Medical Science, University of South Australia, South Australia; ⁵Centre for Heart Rhythm Disorders, University of Adelaide and Royal Adelaide Hospital, Adelaide, South Australia, Australia; ⁶Background: Increased potassium intake has been related to reduced blood pressure (BP) and improved vascular function. Interventional studies have reported inconsistent findings, which may be explained by a threshold required to detect an effect of increased potassium intake on vascular function. The effect of increased dietary potassium on endothelial function remains unknown.

Aim: To determine the effect of increased dietary potassium (70 mmol/day) on endothelial function.

Methods: Thirty-nine healthy men and women (age 32±12 y) completed a randomized cross-over study of 2x6 day diets. The dietary intervention was high potassium (150 mmol/day diet) and usual potassium (80 mmol/day). Measurements of flow mediated dilatation (FMD), BP, pulse wave velocity (PWV), augmentation index (AI) and a fasting blood sample for analysis of intercellular adhesion molecule-1 (ICAM-1), E-selectin and asymmetric dimethylarginine (ADMA) were taken on completion of each intervention. Participants completed 6 day weighed food diaries and a 24 hour urine sample during each diet to estimate adherence to the intervention.

Results: There was no significant difference in FMD between the diets (high potassium 7.02±1.94% vs low potassium 6.56±1.78%, P=0.08). Mean potassium excretion was 92.9±35.0 mmol/day for the high potassium diet and 50.8±30.0 mmol/day for the low potassium diet (P<0.001). When analysis was completed to include compliers to the protocol (n=35, as defined by an increase in urinary potassium excretion greater than 0 mmol/day) FMD was significantly improved following the high potassium diet compared to the low potassium diet (P=0.03). There were no significant differences in systolic BP (P=0.85), diastolic BP (P=0.09), mean arterial pressure (P=0.16), PWV (P=0.67), AI (P=0.31), ICAM-1 (P=0.73), or ADMA (P=0.86) between the interventions. There was a significant reduction in E-selectin following the high potassium diet (Mdn=5.96 ng/ml) vs the low potassium diet (Mdn=8.22 mg/ml; z=-2.64, P=0.008). Potassium intake and potassium excretion were positively correlated following the high potassium and low potassium dietary interventions (r=0.48, P<0.002; and r=0.76, P<0.001, respectively).

Conclusion: An increase in dietary potassium improves endothelial function as assessed by FMD within 1 week in healthy men and women when an increase in potassium is achieved. This suggests potassium intake may have protective effects on vascular function, although the mechanisms for this effect remain unclear.

A-012

DIET QUALITY AND ARTERIAL COMPLIANCE IN PEOPLE WITH TYPE 1 AND TYPE 2 DIABETES

A-013

FENUGREEK EXTRACT IS MORE EFFECTIVE THAN ITS ACTIVE COMPOUND, SAPONINS, IN ATTENUATING ENDOTHELIAL ACTIVATION IN HUMAN ARTERIAL ENDOTHELIAL CELLS

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¹The Centre for Pathology Diagnostic and Research Laboratories (CPDRL) and Institute of Medical Molecular Biotechnology (IMMB), Faculty of Medicine, Universiti Teknologi MARA, Sungai Buloh, Malaysia. ²Background: Inflammation and endothelial dysfunction are key events in the pathogenesis of atherosclerosis. There have been recent advances in identifying cardioprotective properties in natural products. Trigonella foenum graecum, more commonly known as fenugreek, is one compound that has attracted interest. However, to date, there have been few studies on the potential atheroscleroprotective properties of fenugreek and its active class of compounds, saponins.

Aim: To determine the effects of fenugreek crude extract and its saponins on protein and gene expression of endothelial activation biomarkers in stimulated human coronary artery endothelial cells (HCAECs).
Methods: LPS stimulated confluent HCAECs (Lonza, USA) were treated with fenugreek and saponins, respectively. Cells were treated for 48-72 hours, and at 16 hours post-incubation supernatants were tested by enzyme-linked immunosorbent assays (ELISA) (Biobrascence, Inc.). North America) to measure sICAM-1 and sVCAM-1 protein expression. RNA was extracted from the cells for analysis of gene expression.

Results: Treatment with fenugreek reduced sICAM-1 and sVCAM-1 protein: 46.9 vs. 93.8 µg/ml (P=0.029) and 46.9 vs. 93.8 µg/ml (P=0.021), respectively, and gene expression: 93.8, 187 and 375 µg/ml (P=0.03) and 46.9, 93.8, 187 and 375 µg/ml (P<0.04) respectively. In contrast, saponin treatment increased protein expression of sICAM-1: 3.2, 63.5 and 12.5 (P<0.03) with no change in gene expression (P=0.05). There was no effect on sVCAM-1 protein (P=0.05), but a reduction in sVCAM-1 gene expression at high concentration (25.0 µg/ml, P=0.029).

Conclusion: Fenugreek crude extract is more effective than the active class of compound, saponins, in attenuating endothelial activation in HCAEC. This suggested that fenugreek in its natural form has better potential than the active constituent, saponins, as an anti-atherosclerotic agent.

Y CHROMOSOME-LINKED LONG NON-CODING RNA: FUNCTION AND INVOLVEMENT IN DISEASES WITH IMPLICATIONS FOR CORONARY ARTERY DISEASE

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Background: Diabetes is a major risk factor for coronary artery disease (CAD), which is responsible for substantial morbidity and mortality among people with diabetes. The diabetic metabolic environment predisposes to aggressive CAD that causes heart attacks, heart failure and death. However, the pathogenesis of the vascular and myocardial complications of diabetes is not well understood. In this context, studies have demonstrated a positive correlation between increased hepatic free fatty acids (FFAs) in atherosclerosis and CAD. Recently, studies have shown that the human Y chromosome is associated with a greater risk of CAD in men. Moreover, long non-coding RNAs (lncRNAs) have gained attention as a new class of regulatory RNAs involved in cardiovascular function and associated disease. However, the molecular mechanisms implicated are not well defined.

Aims: We aim to identify the Y-specific lncRNAs involved in atherosclerosis and to investigate their role in this disease by an in vitro and in vivo characterization.

Methods: Primers for ten IncRNA transcripts from the Y chromosome annotated sequence were designed and analysed by quantitative PCR (qPCR) in a human tissue panel for differential expression of IncRNA. To create an insulin resistant model, human liver HepG2 cells were treated with 0.3 mM of the FFA palmitate for 24 hours and qPCR was performed on the transcripts.

Results: qPCR measured six IncRNA transcripts expressed in untreated liver tissues and in Hep G2 cells. In Hep G2 cells treated with palmitate, statistical analysis (Student's t-test) determined a significant increase in the expression of two IncRNA transcripts, Inc-KDM5D-41 (P=0.019) and Inc-ZFY-1 (P=0.032).

Conclusion: Given the role of FFA in the pathogenesis of CAD, we propose that IncRNAs in the liver may play a role in regulating metabolic processes implicated in atherosclerosis and thereby participate to the risk of CAD in men.

SAXAGLIPTIN AND CARDIOVASCULAR OUTCOMES IN PATIENTS WITH TYPE 2 DIABETES

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Aim: The aim of the SAVOR-TIMI 53 trial was to evaluate the long-term cardiovascular efficacy and safety of saxagliptin in patients with type 2 diabetes (T2D) at risk for cardiovascular events.

Methods: The study randomly assigned 16,492 patients with T2D who had a history of, or were at risk for, cardiovascular events to receive saxagliptin or placebo and followed them for a median of 2.1 years.

Results: Overall, saxagliptin neither increased nor decreased the risk of the primary endpoint of cardiovascular death, myocardial infarction, or ischemic stroke. The composite secondary end point was balanced, supporting the overall cardiovascular safety. However, there was an unexpected 27% increased relative risk (95% confidence interval) in patients with a history of heart failure or elevated levels of NT-proBNP, that identified patients at an overall increased risk of hHF, although, even in those patients the primary and secondary endpoints were balanced. Saxagliptin improved glycemic control with significantly greater percentage of patients reaching A1C without hypoglycemia in spite of a 30% decrease in initiation of insulin therapy and a 23% reduction of increase in oral hypoglycemic therapy. Saxagliptin significantly reversed or prevented deterioration of microalbuminuria after 1 and 2 years on therapy. Saxagliptin increased major hypoglycemic events in patients treated with sulfonylurea and baseline hba1c <7% without increasing the need for hospitalization or the primary or secondary endpoint. There was no excess hypoglycemia with other background anti-diabetic treatments including insulin. Overall adverse events including adverse events of special interest for anti-diabetic medications as well as incretin-based therapies were similar between saxagliptin and placebo. This DPP4 inhibitor did not increase the overall risk or severity of pancreatitis or show signs of increased risk for pancreatic cancer.

Conclusions: Findings from the SAVOR study expand and clarify the saxagliptin safety profile established in the Phase 2b/3b clinical program, through greater exposure in an older population with longer diabetes duration and multiple risk factors for, or established, cardiovascular disease. The safety and tolerability of saxagliptin use in T2D is supported by the SAVOR results.

A-014

MECHANISMS OF DIABETES-ACCELERATEDATHEROSCLEROSIS

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Background: Diabetes accelerates the formation and progression of atherosclerotic lesions, which likely explains the increased risk of cardiovascular disease in diabetic humans. The accelerated atherosclerosis is driven, at least in part, by the altered function and properties of myeloid cells, namely cells involved and contributing to an increased inflammatory vascular state in atherogenesis. Pro-inflammatory changes in endothelial cells also likely contribute to diabetes-accelerated atherosclerosis.

Aim: To identify cellular and molecular mechanisms of diabetes-accelerated atherosclerosis using mouse models.

Methods and Results: In mouse models of type 1 diabetes, initiation of atherosclerosis is accelerated due to increased accumulation of macrophages in the arterial wall. Based on our recent studies, the enzyme acyl-CoA synthetase 1 (ACS L1), which converts long-chain fatty acids into their acyl-CoA derivatives, has emerged as causal to the enhanced atherosclerosis associated with diabetes. ACSL1 is expressed at higher levels in myeloid cells from diabetic mice, and is induced by inflammatory mediators in these cells. Moreover, deletion of ACSL1 in myeloid cells results in complete protection of these cells from the inflammatory activation associated with diabetes and from early diabetes-accelerated atherosclerosis. Interestingly, ACSL1 deficiency appears to target a pathway that is selectively activated by diabetes. In endothelial cells, ACSL1 is induced by TNF-α, and contributes to TNF-α-induced secretion of the important chemokine CCL2, suggesting that endothelial ACSL1 might also promote atherosclerosis.

Conclusion: Myeloid ACSL1 specifically mediates diabetes-accelerated atherosclerosis in mice. An important question is whether ACSL1 and other factors identified in mouse models play equally important roles in diabetes-accelerated atherosclerosis in humans.

A-016

THE ROLE OF DOMINANT-ACTIVE IDOL IN DIET INDUCED HYPERCHOLESTEROLEMIA AND Atherosclerosis

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Background: Dyslipidemia is a common feature of diabetes and the metabolic syndrome, and can manifest itself as an elevated level of LDL cholesterol. The ES ubiquitin ligase, IDOL (inducible degrader of the LDL receptor, LDLR), is a novel regulator of LDLR-dependent cholesterol uptake, but its mechanism of action, and its influence on plasma cholesterol and atherosclerosis in vivo have not been examined.

Aim: We sought to define the IDOL-LDLR interaction and to examine the consequence of chronic liver-specific expression of a dominant active form of IDOL in mice.

Methods and Results: Through the use of mutational studies, we identified critical residues within the FERM domain of IDOL that bind a newly identified recognition sequence in the cytoplasmic tails of its lipoprotein receptor targets. Furthermore, we expressed a degradation-resistant, dominant-active form of IDOL (sIDOL) in C57Bl/6J mice from the liver-specific albumin promoter (L-sIDOL Tg). L-sIDOL transgenic (Tg) mice were fed a Western diet for 20 or 30 weeks and then analyzed for plasma lipid levels and atherosclerotic lesion formation. L-sIDOL Tg mice demonstrated substantial reductions in hepatic LDLR protein and in increased plasma LDL levels in both Chow and Western diets. Moreover, L-sIDOL mice developed marked atherosclerotic lesions when fed a Western diet, with male mice showing a two-fold greater lesion burden compared to females. qPCR revealed...
elevated mRNA expression of LXR target genes such as ABCA1 and IDOL as well as pro-inflammatory mediators such as TNFα and IL-6 in aortas of Western diet-fed L-SDIL mice.

Conclusion: Our data identify the IDOL–LDLR interaction and suggest that this could potentially be exploited for the pharmacological modulation of lipid metabolism and atherosclerosis. Furthermore, liver-specific expression of dominant active IDOL is associated with hypercholesterolemia and a marked elevation in atherosclerotic lesions. Our results demonstrate that increased activity of the IDOL pathway in the liver can override other LDLR regulatory pathways leading to cardiovascular disease. L-SDIL mice are a robust, dominantly-inherited, diet-inducible model for the study of atherosclerosis.

**A-018**

DIABETES INCREASES RETICULATED PLATELETS DUE TO ENHANCED PROLIFERATION AND EXPANSION OF BONE MARROW MEGAKARYOCYTE PROGENITORS

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**Background:** Diabetes (types 1 and 2) is a major risk factor for cardiovascular disease (CVD) and represents the major cause of mortality in affected patients. Even with current treatments, diabetic patients with CVD have worse outcomes than patients with CVD alone. This is likely explained by the mechanisms contributing to accelerated atherosclerosis in diabetes being different without diabetes. We hypothesized that increased reticulated platelets, which play an important role in atherogenesis, are increased in diabetes by overproduction from the bone marrow (BM).

**Aim:** To investigate the mechanisms contributing to increased reticulated platelets in diabetes.

**Methods:** Wild-type mice were made diabetic with streptozotocin and the number of total and reticulated platelets was measured. To determine the mechanisms for increased platelets we assessed the population of megakaryocyte progenitors (MKPs) in the BM by flow cytometry. We also examined the levels of thrombopoietin (TPO) in the plasma by ELISA. To determine if hematopoietic RAGE was playing a role we performed BM transplant studies comparing wild-type to Rag2-/- BM.

**Results:** We found a significant increase in platelets and reticulated platelets in diabetic mice. This appeared to be due to an over-production as MKPs were expanded and proliferating more in the BM of hyperglycemic mice. This also resulted in more megakaryocytes in the BM. We also found an increase in plasma TPO levels, but no change in the TPO receptor, c-MPL, on any of the BM progenitor cells or circulating platelets, suggesting that the effect was due to increased TPO production from the liver. The expression of TPO is generally increased in diabetes. These findings emphasise the importance of utilizing either non-BM- or BM-derived RAGE in the development of therapeutics to target the prevention and treatment of diabetes-associated atherosclerosis.

**A-020**

EFFECT OF NIACIN ON TRIGLYCERIDE-RICH LIPOPROTEIN APOLIPROPROTEIN B-48 KINETICS IN TYPE 2 DIABETIC SUBJECTS ON A STATIN

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**Background:** Type 2 diabetic subjects often have hypertriglyceridaemia and an increased concentration of apolipoprotein B-48 (apoB-48) in the circulation, particularly in the post-prandial period. There is an accumulating body of evidence to suggest that apoB-48 plays a central role in the development of atherosclerosis. Statins are the frontline therapy to reduce cardiovascular risk. However, a large residual risk still remains. This residual risk suggests that additional therapeutic interventions may be required to further reduce cardiovascular disease (CVD) risk.

**Aim:** To investigate the effect of niacin on the metabolism of triglyceride rich lipoprotein (TRLs) apoB-48 in men with type 2 diabetes on a background of statin therapy.

**Methods:** Twelve type 2 diabetic men were recruited for this randomized, cross-over design study. Patients required a statin-treated low density lipoprotein (LDL) cholesterol of less than 2.5 mmol/L to enter the trial. Patients were then randomized to rosuvastatin alone or rosuvastatin plus niacin (titrated up from 1 to 2 g daily) for a period of 12 weeks and then were crossed over to the alternate therapy with a 3-week washout period in between. Metabolic studies were performed at the end of each treatment period. A bolus intravenous infusion of D3-leucine was administered as subjects consumed a standardized high-fat liquid meal. Blood samples were collected over 24 hours and TRL apoB-48 tracer/trace ratios were measured using gas chromatography-mass spectrometry. Kinetic parameters, including fractional catabolic rate (FCR) and production rate (PR), were derived using a multicompartamental model.

**Results:** Niacin significantly reduced triglyceride, plasma cholesterol, LDL cholesterol and apoB (all P<0.005). TRL apoB-48 concentration was lower with niacin (P=2.2±1.98 vs 5.48±1.14 mmol/L; P=0.03). ApoB-48 FCR was not altered with niacin (8.7±1.04 vs 9.17±1.26 pools/day; P=0.79). Basal apoB-48 PR (3.21±0.34 vs 2.5±0.31 mg/kg/day; P=0.04) and postprandial apoB-48 PR were significantly lower (1.35±0.19 vs 0.84±0.12 mg/kg; P=0.02) on niacin.

**Conclusion:** Niacin reduces TRL apoB-48 concentration by lowering basal and postprandial apoB-48 PR. This effect on apoB-48 metabolism may be beneficial for reducing atherogenic postprandial TRL particles and may provide CVD risk benefit to type 2 diabetic patients.

**A-021**

NON-LIPID CARDIOVASCULAR RISK FACTORS IN FAMILIAL HYPERCHOLESTEROLEMIA: A CROSS-SECTIONAL STUDY IN WESTERN AUSTRALIA

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**Background:** Familial hypercholesterolemia (FH) is classified due to LDL receptor mutations that result in marked hypercholesterolemia and premature coronary artery disease (CAD). The contribution of other cardiovascular (CV) risk factors in FH is unclear.

**Aim:** To examine the relationship between non-lipid CV risk factors and CAD in FH.

**Methods:** A cross-sectional study of 384 phenotypically (20%) and genotypically (80%) defined FH (211 women and 173 men, age 47.9±15.3 years, BMI 27.1±5.1 kg/m2, Dutch Lipid Network Criteria score >9) from the Western Australia cascade screening program was conducted. The CV risk factors included smoking, diabetes mellitus, hypertension (BP >140/90 mmHg) and obesity (BMI >30 kg/m2). Logistic regression analysis was used to study the relationship between non-lipid CV risk factors and CAD in these patients. CAD was defined as a history of myocardial infarction, or coronary revascularization.

**Results:** The prevalence of CV risk factors were: current/ex-smokers 49%, obesity 21%, hypertension 26%, low HDL-cholesterol 29%, type 2 diabetes 9%, high LDL-cholesterol 94% and previous CAD 20% (myocardial infarction 12%, angioplasty 14%, bypass graft 6%). In univariate logistic regression analysis, history of hypertension (OR 5.25, P<0.0001) and type 2 diabetes (OR 5.05, P<0.0001) were significantly associated with history of CAD in FH patients. Hypertension and diabetes remained significant determinants of CAD in FH patients in multivariate logistic regression analysis adjusting for age and gender (OR 3.24 and 2.88, P<0.001 and P=0.01, respectively).

**Conclusion:** The spectrum of modifiable CV risk factors exists beyond hypercholesterolemia in patients with FH. Aggressive management of all CAD risk factors in FH patients is indicated.

**A-022**

ASSOCIATION BETWEEN SKELETAL MUSCLE FAT CONTENT AND VERY-LOW-DETERITY LIPOPROTEIN-APOLIPROPROTEIN B-100 TRANSPORT IN OBESITY: EFFECT OF WEIGHT LOSS

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Background: Ectopic deposition of fat in skeletal muscle is a feature of metabolic syndrome, but its specific association with dyslipidemia remains unclear. Although the overproduction of very-low-density lipoprotein (VLDL)-apolipoprotein (apo) B-100 may increase deposition of triglycerides in skeletal muscle, this metabolic process may vary with the degree of insulin resistance.

Aim: To examine the association between skeletal muscle fat content and VLDL-apoB-100 kinetics, and the corresponding responses to weight loss in obese subjects.

Methods: Fat content of liver, abdomen and skeletal muscle was measured by magnetic resonance techniques and VLDL-apoB-100 kinetics were assessed using stable isotope tracers in 25 obese subjects. Kinetic parameters were derived using a multicompartimental model.

Results: In univariate analysis (n=25), skeletal muscle fat content was significantly (P<0.05 in all) associated with body weight (r=0.415), visceral fat area at L3 vertebra (r=0.531), energy intake (r=0.531), plasma non-esterified fatty acid (r=0.428) and glucose concentrations (r=0.477). In obese subjects who were insulin sensitive (HOMA score <2.5), skeletal muscle fat content was significantly associated with hepatic fat content (r=0.636), energy intake (r=0.684), plasma triglyceride (r=0.644), apoB-100 (r=0.529), glucose (r=0.622), VLDL-apoB-100 (r=0.390) and fractional catabolic rate (r=0.581) and VLDL-apoB-100 secretion rate (r=0.607). These associations were not found in obese subjects who were insulin resistant (HOMA score >2.5). Of the 25 subjects, 10 underwent a 16-week weight loss program. A low fat diet achieved significant reduction (P<0.05 in all) in body weight, BMI, visceral and subcutaneous fat areas, liver and skeletal muscle fat, energy intake, triglyceride, Insulin, HOMA score, retinal binding protein-4, VLDL-apoB100 concentrations and VLDL-apoB100 secretion rate. There was a significant increase (P<0.05) in plasma adiponectin concentration. The percentage reduction of skeletal muscle fat with weight loss was significantly associated with a corresponding fall in VLDL-apoB100 concentration (r=0.770; P=0.009) and VLDL-apoB100 secretion (r=0.682; P=0.000).

Conclusion: This study demonstrates for the first time the direct association between skeletal muscle fat content and VLDL-apoB-100 transport. Furthermore, with weight loss, the reduction in skeletal muscle fat was associated with lower rates of VLDL-apoB100 secretion.

A-023

PLASMA PROPOTEIN CONVERTASE SUBSTITUTE TILSIN/KEXIN TYPE 9: A MARKER OF APOLIPOPROTEIN B-48 CATABOLISM IN OBESITY

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Background: Postprandial lipemia, characterized by hypertriglyceridemia due to elevated plasma chylomicron apolipoprotein (apo) B-48 concentrations, contributes to the increased risk of cardiovascular disease in obesity. Proprotein convertase substitute/kexin type 9 (PCSK9) is a secreted protease that mediates degradation of low-density lipoprotein (LDL) receptor. Recent evidence also suggests that PCSK9 could play a critical role in the regulation of chylomicron metabolism.

Aim: To investigate the association between plasma PCSK9 concentration and apoB-48 metabolism in obesity.

Methods: Seventeen obese subjects (9 men and 8 women, age 59±6 years, BMI 33±6 kg/m²) were given an oral fat load. ApoB-48 tracer/tracer ratios were measured after intravenous d3-leucine administration using gas chromatography-mass spectrometry. ApoB-48 fractional catabolic rate (FCR) and secretion rate were derived using a non-steady state multicompartmental model that describes the non-steady state post-prandial metabolism of apoB-48. This association appears to be independent of age, obesity, triglyceride, insulin resistance and energy intake.

Results: There was lower CRP (3.0±3.7 vs. 25.5±10.9; P=0.02), SMAD (3.9±3.3 vs. 12.2±4.1; P=0.04) and MPM-12 (8.3±4.3 vs. 10.1±3.3; P=0.01) tissue expression in TEMF-treated groups compared to placebo. There was no difference in tissue expression of IL-6 and ICAM-1, and atherosclerotic lesions between TEMF and placebo groups.

A-025

RS12718465 SINGLE NUCLEOTIDE POLYMORPHISM IN EXON 3 OF APOA1 GENE IS ASSOCIATED WITH LOW HIGH-DENSITY LIPOPROTEIN SUBJECTS


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Background: Coronary artery disease (CAD) is the major cause of death worldwide. High density lipoprotein cholesterol (HDL-c) is a negative risk factor for CAD. Apolipoprotein A1 (ApoA1) gene polymorphisms impact responses of endogenous VLDL and the main exoc sole excision encoding for 243 amino acids. Genetic variation of ApoA1 may contribute to low HDL-c levels. However, its role in causing low HDL-c levels in South East Asian is unclear.

Aim: To identify and characterize ApoA1 single nucleotide polymorphisms (SNPs) in Malaysia.

Methods: Sixty-eight subjects (37 females, HDL-c ≤0.70 mmol/L and 31 males, HDL-c ≤0.65 mmol/L, based on the 2.5% lower cut-off of normal population distribution) and 68 age-, gender- and ethnicity-matched controls (39 females, HDL-c ≥1.3 mmol/L and 29 males, HDL-c ≥1.0 mmol/L) were recruited for this study. Whole blood samples were collected for DNA extraction. ApoA1 gene amplification was carried out by polymerase chain reaction. Amplified DNA fragments were sequenced. Confirmation of the SNPs was measured using Mega 5.1.

Results: DNA sequencing of the ApoA1 gene showed seven SNPs; one SNP (rs12718465) was found in 6/68 case but not in controls, there being a significant association of this SNP with low HDL-c levels (P=0.03) with an odds ratio of 6.58. This variant has been reported previously to affect HDL level.

Conclusion: The ApoA1 SNP rs12718465 is associated with low HDL levels in a Malaysian cohort. Further studies of larger cohorts are required to explore these associations since they could be responsible for enhanced CAD prevalence, especially among those without other risk factors.

A-026

TOCOTRIENOL-ENRICHED MIXED FRACTION SUPPLEMENTATION REDUCES INFLAMMATION AND PLAQUE INSTABILITY IN RABBITS WITH EXPERIMENTALLY INDUCED EARLY ATHEROSCLEROSIS

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Aim: To determine the in vivo effects of TEMF on inflammation and plaque stability in early atherosclerosis.

Methods: Ten New Zealand white rabbits were randomized into two groups and fed with 1% high cholesterol diet (HCD) for two weeks to induce early atherosclerosis, followed by normal diet for another eight weeks. Intervention with either (i) TEMF (15 mg/kg body weight; tocotrienol—tocopherol ratio = 70:30%) or (ii) placebo were given only after HCD commencement at week 2. At the end of the study, aortas were taken and examined for atherosclerotic lesions using Sudan IV staining. Further evaluation on inflammatory markers, i.e., interleukin-6 (IL-6), C-reactive protein (CRP), intercellular adhesion molecules-1 (ICAM-1), smooth muscle actin (SMA) and matrix metalloproteinase-12 (MMP-12) expression in the tunica intima were done by immunohistochemistry.

Results: There was lower CRP (3.0±0.66 vs. 25.5±10.9; P=0.02), SMA (3.9±3.3 vs. 12.2±4.1; P=0.04) and MPM-12 (8.3±4.3 vs. 10.1±3.3; P=0.01) tissue expression in TEMF-treated groups compared to placebo. There was no difference in tissue expression of IL-6 and ICAM-1, and atherosclerotic lesions between TEMF and placebo groups.
Conclusions: TEMF treatment in early atherosclerosis reduces tissue inflammatory biomarkers and plaque instability, despite an absence of an effect on the atherosclerotic lesions.

A-027

AN EXPLORATORY STUDY OF SPHINGOLIPIDIC ALTERATIONS IN PLASMA AND LIPOPROTEINS IN OVERWEIGHT MEN WITH VARYING DEGREES OF DYSGLYCEMIA


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Background: Alterations in plasma sphingolipids have been associated with the development of insulin resistance and type 2 diabetes (T2D). The effects of different degrees of abnormal glycemia on plasma and lipoprotein sphingolipid composition have not been comprehensively examined.

Aim: To investigate changes in the sphingolipid profile in plasma and lipoproteins in dyslipidemic, overweight men with normoglycemia (NGT), impaired fasting glucose (IFG), impaired glucose tolerance (IGT), and T2D.

Methods: Sphingolipid profiling in plasma and lipoproteins were carried out in 24 dyslipidemic, overweight, and age-matched men with normal glucose tolerance (n=8), impaired fasting glucose (n=8), impaired glucose tolerance (n=8), or type-2 diabetes (n=6). Lipoproteins were isolated by fast performance liquid chromatography. Sphingolipids were quantified by tandem mass spectrometry. Plasma cholesterol efflux was assessed by 3H-cholesterol in THP-1 macrophages.

Results: Compared with NGT subjects, total (+43%; P<0.004) and individual plasma ceramides were significantly elevated in T2D subjects. Total and individual high-density lipoprotein (HDL)-sphingomyelin (SM) species were significantly lower in T2D subjects compared with NGT (total HDL-SM: –30%; P=0.02) and IGT subjects (total HDL-SM: –21%; P=0.03). No statistically significant group differences were found with sphingolipids in VLDL and LDL. After adjustment for HDL-cholesterol, cholesterol efflux in T2D subjects was significantly higher compared with non-T2D subjects (+27%; P=0.01) and with NGT (+43%; P=0.08). 

Conclusion: Plasma- and HDL-sphingolipids are differentially altered with dysglycemia. Lowered HDL-SM enrichment was accompanied by increased plasma cholesterol efflux in T2D and may reflect a compensatory mechanism to counter the dyslipidemia associated with T2D.

A-028

DOSE-DEPENDENT EFFECTS OF ROSUVASTATIN ON THE PLASMA SPHINGOLIPIDOME IN THE METABOLIC SYNDROME

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Background: 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase inhibitors (statins) are established low density lipoprotein (LDL)-cholesterol lowering agents. Statins may, however, also exert effects on the plasma sphingolipidome beyond LDL-cholesterol lowering.

Aim: To investigate the dose-dependent effects of rosuvastatin on plasma sphingolipids in men with the metabolic syndrome.

Methods: Men with the metabolic syndrome (n=12) were studied in a randomized, double-blind, triple-crossover trial involving a 5 week treatment period with placebo or rosuvastatin 5 mg/day (R10) or 20 mg/day (R40) with 2 week wash-outs between treatments. Plasma sphingolipid profiling was determined by liquid chromatography electrospray ionization tandem mass spectrometry.

Results: Rosuvastatin at 10 mg/day (R10) and 40 mg/day (R40) significantly (P<0.001 unless stated otherwise) lowered plasma cholesterol (~34% change with R10, ~42% change with R40) and LDL-cholesterol (~49% with R10 and ~57% with R40) and triglyceride (~24% with R10, P=0.03; and ~42% with R40). Compared with placebo, R10 and R40 significantly decreased plasma levels of total ceramide (~33% with R10 and ~37% with R40), sphingomyelin (~27% with R10 and ~31% with R40), and monoacylglycerol (~40% with R10 and ~47% with R40), diacylglycerol (~31% with R10 and ~34% with R40), triacylglycerol (~29% with R10 and ~31% with R40), and GM1 ganglioside (~29% with R10 and ~26% with R40). Reduction in plasma ceramide was associated with a reduction in very-low-density lipoprotein (VLDL) apolipoprotein (apo) B-100 fractional catabolism independent of changes in LDL-cholesterol with high-dose rosuvastatin.

Conclusion: Rosuvastatin is effective at achieving reductions in total and individual plasma sphingolipids in metabolic syndrome men with evidence of dose-dependent effects. These changes relate to the fractional catabolism of VLDL apoB-100 and were independent of LDL-cholesterol lowering.

A-029

ENHANCED ENDOTHELIAL ACTIVATION IN HUMAN SUBJECTS WITH LOW CONCENTRATION OF HIGH DENSITY LIPOPROTEIN


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Background: Soluble vascular cell adhesion molecule I (sVCAM-I), intercellular cell adhesion molecule I (sICAM-I), and E-selectin are biomarkers reflecting endothelial activation. Endothelial activation has been established as one of the key events in the initiation and progression of atherosclerosis. High-density lipoprotein cholesterol (HDL-c) is a negative risk factor for atherosclerosis, which plays a vital role in reverse cholesterol transport. The role of HDL-c in endothelial activation, however, remains unclear.

Aim: To compare the serum sVCAM-I, sICAM-I and E-selectin concentrations between subjects with low HDL-c and controls.

Methods: 177 subjects (72 males and 105 females aged 44.0 ±11.35 years) with low HDL-c levels (HDL-c <1.0 mmol/L and <1.3 mmol/L for males and females, respectively) and 209 age-, gender-, ethnicity-, smoking-, diabetes- and hypertension-matched normal controls were recruited. Fasting blood samples were collected. Lipid profiles were measured using automated analyser (Cobas Integra 400, Roche, Germany). Enzyme-linked immunosorbent assays (ELISA) were performed to measure the concentrations of sVCAM-I, sICAM-I and E-selectin.

Results: We found higher sVCAM-I, sICAM-I and E-selectin concentrations in low HDL-c patients, than controls: 726 ±225E vs 535 ±203E ng/ml (P=0.01); 798 ±33SE vs 685 ±21SE ng/ml (P=0.01); and 47.7 ±7.0 vs 28.3 ±2.3 ng/ml (P=0.01), respectively.

Conclusion: These findings suggest there is increased endothelial activation in subjects with low HDL-c levels and which in part may contribute to enhanced atherosclerosis.

A-030

MODULATION OF ENDOTHELIAL CELL FUNCTION BY THE MYELOPEROXIDASE-DERIVED OXIDANT HYPOCHLOROUS ACID (HOCl) AND ITS CONTRIBUTION TO ATHEROSCLEROSIS

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Background: Myeloperoxidase (MPO) is a haem enzyme released by activated phagocytes under inflammatory conditions. MPO forms reactive oxidants that play an important role in the human immune system. Hypochlorous acid (HOCl) is the major oxidant produced by MPO under normal physiological conditions. Although HOCl is a potent bactericidal agent, excessive or misplaced production of HOCl has been linked to tissue damage and the progression of many diseases, including atherosclerosis. MPO is also an independent risk factor for the development of coronary artery disease and a powerful prognostic agent predicting outcome in patients with chest pain or myocardial infarction.

Aim: In this study, we examine the cellular pathways responsible for the induction of endothelial cell death and dysfunction on exposure of cells to HOCl.

Methods: Primary human coronary artery endothelial cells (HCAEC) were exposed to pathophysiological levels of HOCl. Cellular targets were assessed by a proteomics approach, molecular pathway expression was analysed by real-time PCR and Western blotting, and the extent of cell death was measured by flow cytometry.

Results: Exposure of HCAEC to HOCl resulted in a dose-dependent increase in cell death, with programmed cell death (apoptosis) observed at low doses and necrosis predominating at higher doses. Proteins containing free Cys (thiol) residues are important targets for HOCl, with protein disulphide isomerase (PD-I), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), thioredoxin-related protein and galectin-1 found to be particularly sensitive to oxidation. This results in modulation of enzyme function, with rapid inactivation of GAPDH observed after HOCl treatment. HOCl exposure also induced several key transcription factors involved in inflammatory pathways, including activating transcription factor 3 (ATF3), activator protein 1 (AP-1), early growth response 1 (Egr-1), and CCAT/enhancer binding protein homologous protein (CHOP). Expression of these molecules occurred concurrently with the induction of mediators of inflammation such as TNFα, interleukin 8 (IL8), and monocytic chemotactic protein-1 (MCP-1).

Conclusion: Targeting cellular thiol-containing proteins and activation by HOCl of the transcription factors AP-1, Egr-1, and CHOP results in endothelial cell death and the propagation of inflammation, which may accelerate atherosclerosis and contribute to the development of clinically-relevant lesions.

A-031

ATORVASTATIN PLUS OMEGA-3 FATTY ACID ETHYL ESTER DECREASES VERY-LOW-DENSITY LIPOPROTEIN TRIGLYCERIDE PRODUCTION IN INSULIN RESISTANT OBSESE MEN

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Background: Abnormal very-low-density lipoprotein (VLDL)-triglyceride (TG) metabolism underscores the hypertglycemia in obesity, insulin resistant men and contributes to increased cardiovascular disease (CVD) risk. Statins reduce CVD risk by decreasing low-density lipoprotein (LDL)-cholesterol, but their effects on plasma TGs are modest. Supplementing with high dose omega-3 fatty acid ethyl esters (3-FAEES) may achieve a greater reduction in plasma TG concentration and potentially attenuate residual CVD risk.

Aim: To test the effect of atorvastatin and atorvastatin plus -3 FAEEs on VLDL-TG metabolism in obese, insulin resistant men.

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Methods: We carried out a 6-week randomized, placebo-controlled study to examine the effect of atorvastatin (40 mg/day) and atorvastatin plus 3 FAEEs (4/gday) on VLDL-TG metabolism in 36 insulin resistant, obese men. VLDL-TG kinetics were determined using d3-glycerol, gas chromatography-mass spectrometry, and compartmental modeling.

Results: Compared with placebo, atorvastatin significantly decreased VLDL-TG concentration (−40%; *P<0.001) by increasing VLDL-TG fractional catabolic rate (FCR) (+47%; *P<0.01). Atorvastatin plus 3 FAEEs lowered VLDL-TG concentration to a greater degree compared with placebo (−46%; *P<0.01) or atorvastatin monotherapy (−13%; *P=0.04). This was achieved by a reduction in VLDL-TG production rate (PR) compared with placebo (−32%; *P=0.008) or atorvastatin (−20%; *P=0.03), as well as a reciprocal increase in VLDL-TG FCR (+42%; *P<0.05) compared with placebo.

Conclusion: In insulin resistant, dyslipidemic, obese men, atorvastatin improves VLDL-TG metabolism by increasing VLDL-TG FCR. The addition of 4 g/day -3 FAEE to statin therapy provides further TG-lowering by reducing VLDL-TG PR.

A-032

THERAPEUTIC USE OF ENOS/CAVEOLIN-1 ANTAGONISTIC PEPTIDES FOR DIABETES-ASSOCIATED ATHEROSCLEROSIS

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Background: Diabetes is associated with an increased risk for the development of atherosclerosis, mainly due to the reduction in endothelial function, which is characterized by decreased nitric oxide (NO) bioavailability. Endothelial nitric oxide synthase (eNOS), the enzyme responsible for the production of NO, is negatively regulated through its association with caveolin-1, the major coat protein of caveolae. We have recently shown that a mutant Cavelin-1-derived peptide (CavNoxin) increases NO release and bioavailability by antagonizing the binding of eNOS to caveolin-1. The upregulation of NO production is therapeutically relevant in atherosclerosis we hypothesized that CavNoxin can attenuate the progression of diabetes-associated atherosclerosis.

Aim: To examine whether antagonizing the eNOS/caveolin-1 interaction in diabetes might be a potentially novel and unexplored antiatherosclerotic therapeutic strategy.

Methods: 8-week old apolipoprotein E (ApoE) knockout mice were rendered diabetic with the diabeticogenic drug, streptozotocin. At 10 weeks of age, diabetic mice were injected with either CavNoxin (2.5 mg/kg and 5.0 mg/kg) or vehicle once every 3 days for 14 weeks.

Results: Diabetes led to an increase in atherosclerotic lesions throughout the aorta (total plaque: 1.5% in non-diabetic vs 11% in diabetic mice; *P<0.001) and aortic sinus, which was significantly attenuated with CavNoxin treatment (total plaque: 5%; *P<0.01 vs diabetic). The reduction in atherosclerotic lesions was associated with decreased oxidative stress, as assessed by DHE and 4-HNE staining, and expression of pro-atherogenic mediators, such as VCAM-1 (mRNA fold induction: 2.8 in diabetic vs 1.5 in diabetic + CavNoxin; *P=0.05) and MCP-1 (mRNA fold induction: 11 in diabetic vs 4.5 in diabetic + CavNoxin; *P<0.05). In cultured endothelial cells grown in high glucose and diabetic aortas perfused with whole blood, CavNoxin treatment led to a significant reduction of leukocyte/monocyte adherence (*P<0.05 and *P<0.001, respectively).

Conclusion: These data are the first to show a positive effect of eNOS/caveolin-1 antagonism on diabetes-associated atherosclerosis. Since superoxide is an NO scavenger, these data suggest that CavNoxin mediates its antiatherogenic effect by increasing NO bioavailability.

A-033

A NOVEL MOUSE MODEL THAT REFLECTS HUMAN ATHEROSCLEROTIC PLAQUE INSTABILITY IS A UNIQUE TOOL FOR DRUG TESTING AND MECHANISTIC DISCOVERIES

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Background: The high morbidity/mortality of atherosclerosis is typically precipitated by plaque rupture and consequent thrombosis. However, research on underlying mechanisms and therapeutic approaches is significantly hampered by the lack of animal models that reproduce plaque instability observed in humans.

Aim: To generate a mouse model that reflects human plaque instability/rupture.

Methods: Typical hemodynamic conditions that contribute to the development of vulnerable, unstable atherosclerotic plaques are low shear stress and high tension stress. Based on computational fluid dynamics modeling, we developed a mouse model that imitates these hemodynamic conditions. A tandem stenosis was applied to the carotid artery of mice, which were fed a high-fat diet.

Results: At 7 weeks postoperatively, we observed intraplaque hemorrhage in ~50% of mice, as well as disruption of fibrous cap, intraluminal thrombosis, neovascularization and further characteristics typically seen in human unstable atherosclerotic plaques. Administration of atorvastatin was associated with plaque stabilization and down-regulation of MCP-1 and ubiquitin. Microarray profiling of mRNA and microRNA, in particular its combined analysis, demonstrated major differences in the hierarchical clustering of genes and microRNAs between non-atherosclerotic arteries, stable and unstable plaques and allowed the identification of distinct genes/microRNAs, potentially representing novel therapeutic targets for plaque stabilization. The feasibility of the animal model described as a diagnostic tool was established in a pilot approach, identifying ADAMTS4 and miR-322 as potential pathogenic factors of plaque instability in mice. The involvement in plaque instability was validated in human atherosclerotic plaques.

Conclusion: The newly described mouse model reflects human atherosclerotic plaque instability/rupture and represents a unique discovery tool that allows the identification of distinctly expressed genes and microRNAs that are linked to plaque instability. It holds promise towards the development and testing of therapeutic strategies aimed at preventing plaque rupture.

A-034

TRAIL-DEFICIENCY ACCELERATES VASCULAR CALCIFICATION IN ATHEROSCLEROSIS VIA MODULATION OF RANKL

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Background: An increasingly recognized risk factor for cardiovascular disease is vascular calcification. The triad cytokine system of osteoprotegrin (OPG), receptor activator of nuclear factor-κB ligand (RANKL) and its receptor RANK control bone homeostasis. This system has recently been implicated in vascular calcification. Osteoclastogenesis is initiated by RANKL binding to RANK expressed on osteoclasts. RANKL is also able to bind its soluble receptor, OPG, which inhibits differentiation, maturation and survival of osteoclasts, impeding bone mineralization. TNF-α-related apoptosis-inducing ligand (TRAIL) is a second ligand for OPG and is protective for atherosclerosis, yet the role of TRAIL in vascular calcification is unclear.

Aim: To identify the involvement of TRAIL in vascular calcification in arteries in vivo using TRAIL–/–ApoE–/– and ApoE–/– mice.

Methods: Calcification of vascular smooth muscle cells (VSMCs) was measured using an alizarin red-based assay. Calcification in vivo was assessed in TRAIL–/–ApoE–/– and ApoE–/– mice placed on a high fat diet for 12 and 20 weeks. mRNA and inflammatory markers were measured by standard techniques.

Results: In vitro, TRAIL dose-dependently inhibited calcium-induced calcification of human VSMCs. Correspondingly, murine TRAIL–/–VSMCs demonstrated accelerated calcification, compared to wild-type (WT) cells, when induced by multiple concentrations of calcium. Wild-type cells exposed to calcium had significantly elevated mRNA expression of RANKL, a pro-calcific factor, while OPG and TRAIL were inhibited. At 12 weeks on high fat diet, calcific arteries from TRAIL–/–ApoE–/– mice showed increased numbers of chondrocyte-like cells, whereas by 20 week TRAIL–/–ApoE–/– aortas displayed significantly increased calcification. 12 week TRAIL–/–ApoE–/– aortas had increased expression of mRNA for the osteochondrogenic marker, collagen II, as well as cellular RANKL, with no change in circulating RANK or OPG. They also displayed altered expression of inflammatory markers regulating bone, including increased IL-1α and PPAR-α.

Conclusion: This study provides the first evidence that TRAIL deficiency leads to accelerated carotid vascular calcification in arteries in vivo. TRAIL plays an important role in the regulation of RANKL and inflammatory markers mediating bone turnover in the vasculature in vivo. We also demonstrate that TRAIL has a protective role in vascular calcification in vivo.

A-035

PHARMACOLOGICAL INHIBITION OF NADPH OXIDASE (NOX)- DERIVED ROS ATTENUATES IMMUNE-INFLAMMATORY RESPONSES AND ACCELERATED ATHEROSCLEROSIS IN DIABETES

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Background: Enhanced infiltration of inflammatory-immune cells and heightened vascular oxidative stress are key processes driving atherosclerotic development in diabetes. Growing evidence demonstrates a central role for NADPH (Nox) isozymes in pathological production of reactive oxygen species (ROS) in the vasculature.

Aim: The aim of this study was to examine the role of Nox-derived ROS in pro-inflammation and pro-oxidant responses involved in diabetes-associated atherosclerosis.

Methods: Diabetes was induced in ApoE deficient (ApoE–/–) mice by 5 injections of streptozotocin (55 mg/kg/day). The Nox1/4 dual inhibitor, GKT137831, was administered to diabetic and non-diabetic groups at a dose of 60 mg/kg/day by gavage for 10 weeks. At study completion, aortic sinuses were isolated and analysed for atherosclerotic plaque area, immune cell infiltration and gene expression.

Results: Induction of diabetes in ApoE–/– mice significantly increased atherosclerotic plaque area in the aortic sinus (63%) and necrotic core size in association with elevated MCP-1 expression and increased lesional accumulation of CD4+ CD8+ and CD211c+ cells. In addition, diabetic mice displayed increased vascular superoxide as measured by dihydrodichlorodihydrofluorescein (DHE) fluorescence, which correlated with elevations in Nox1 and Nox4 gene expression in the aortic sinus. Pharmacological inhibition of Nox-derived ROS using
GKT137831 attenuated the diabetes-mediated changes in atherosclerosis plaque size and phenotype in the Apoe-/- mice. 

Conclusions: Accelerated atherosclerosis development in the aortic sinus of Apoe-/- mice was associated with elevated production of N ox-derived ROS and increased accumulation of inflammatory cells typically involved in adaptive immune responses. Treatment with GKT137831 ameliorated these diabetes-induced pro-atherogenic responses, thus supporting a central role of Nox1 and Nox4 isoforms in diabetes-associated atherosclerosis.

VANIN-1, A NOVEL GENE THAT INFLUENCES CHOLESTEROL HOMESTASIS


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Background: A low level serum of high density lipoprotein cholesterol (HDL-C) is a strong independent risk factor for development of cardiovascular disease (CVD). There are, however, limited therapeutic options to increase HDL-C, leading to an urgent need for therapies. In our prior work, we combined genomic, transcriptomic and functional genetic analyses to identify the gene for vanin-1 (VNN1) as a novel gene whose expression shows a significant correlation with human HDL-C levels.

Aim: To validate the association between HDL-cholesterol and VNN1 expression using in vitro cellular models and to identify the mechanisms of action of vanin-1 on HDL cholesterol metabolism.

Methods: To investigate how vanin-1 affects HDL cholesterol, experiments that included cholesterol efflux assays and confocal microscopy were performed on in vitro cellular models where VNN1 was either silenced or over-expressed.

Results: Over-expression of VNN1 significantly increased ApoA1-mediated cholesterol efflux in human liver (70% increase; P<0.015), colorectal (70% increase; P<0.015) and small intestine (60% increase; P<0.023) cells. In contrast, siRNA-mediated silencing of VNN1 mRNA significantly decreased ApoA1-mediated cholesterol efflux. Similar trends were observed with HDL-mediated cholesterol efflux. siRNA-mediated knockdown of ABCA1 mRNA prevented VNN1 overexpression from significantly increasing cholesterol efflux in HepG2 cells, suggesting that the effect of vanin-1 on cholesterol efflux is reliant on the ABCA1 transporter. Furthermore, suppression and over-expression of VNN1 mRNA affected the distribution of late endosomes within HepG2 cells.

Conclusion: Taken together, these data suggest that vanin-1 is an important in vivo regulator of cholesterol efflux in various human cell types. The study also suggests that the effects of vanin-1 on cholesterol efflux are dependent on the ABCA1 transporter. We propose that vanin-1 may influence cholesterol efflux and metabolism by regulating late endosome distribution. Our data suggest that vanin-1 may be an important regulator of HDL-C and could be a potential candidate for the development of new HDL-raising agents.

IMAGING OF ADVANCED ATHEROSCLEROTIC PLAQUE BY HIGH RESOLUTION, MULTI-ENERGY X-RAY COMPUTER TOMOGRAPHY


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Background: Cardiovascular disease (CVD) is usually symptom free until atherosclerotic plaques have become large enough either to restrict blood flow or to rupture, triggering clotting and occlusion of the artery. Under ultrasound, MRI or CT imaging, preclinical atherosclerotic plaques are usually too small to be seen, since they are less than 2 mm thick. As a result, treatment of CVD often does not occur till after the plaque has become structurally complicated and is causing significant clinical harm. Clinicians treat the signs and symptoms of CVD, such as high plasma cholesterol, rather than the actual plaque growth. Imaging of preclinical plaque requires the development of high-resolution scanners with low radiation dose, which are capable of distinguishing between different types of tissue. One such approach is to measure the X-ray energy of the individual photons that have passed through the plaque tissue.

Aim: To describe a novel method for detection of preclinical plaque from human carotid arteries.

Methods: Using a MARS scanner designed and constructed at the Universities of Otago and Canterbury, we image excised plaque from human carotid arteries in the human CT energy range (20–120 kV). The X-ray camera within the MARS scanner contains a CERN-designed Medipix3.1 X-ray detector chip, which measures the location, timing and energy of each single X-ray photon striking any one of the 16,512 pixels on the sensor layer, which in these experiments was CdTe. This unique imaging system allowed us to simultaneously collect X-ray attenuation data from the plaque tissue in 4 separate energy bins. Images were reconstructed and regions of tissue were distinguished by material decomposition using the known composition of X-ray attenuation within each voxel.

Results: Multi-energy imaging using the MARS scanner has allowed us to distinguish lipid-rich regions from water-rich and calcium-rich regions within carotid plaque. The chemical characteristics of these regions were confirmed by histological staining and comparisons to phantom materials of known composition. The image analysis was able to show calcium deposition occurring in sheets near the basement membrane of the arterial wall.

Conclusion: High resolution, multi-energy X-ray computer tomography can distinguish pre-clinical plaque excised from carotid arteries. This technology is being further developed to allow rapid imaging of live small animals to enable the analysis of plaque development over time in cholesterol fed animal models.

TRAK2, A NOVEL REGULATOR OF CHOLESTEROL EFFLUX AND HDL BIOGENESIS


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Background: An improved understanding of the genes that influence HDL-cholesterol abundance is required urgently in order to enable development of effective HDL-cholesterol-raising therapies. We previously employed genetic and transcriptomic analyses of human cohorts to identify a novel association and negative correlation between TRAK2 and HDL-cholesterol levels. As TRAK2 had not been implicated previously in lipid metabolism, the mechanism underlying the association of TRAK2 with HDL-cholesterol was not known.

Aim: To characterize the biological mechanism underlying the in vivo association between TRAK2 and HDL-cholesterol abundance.

Methods: In vitro cellular models of siRNA-mediated silencing were used to investigate the role of TRAK2 in pathways that regulate HDL-cholesterol metabolism.

Results: siRNA-mediated silencing of TRAK2 mRNA significantly increased cholesterol efflux to apoA1 and HDL in human liver HepG2 cells by 60% and 40%, respectively. TRAK2 mRNA silencing in HepG2 cells significantly increased the expression of the key cholesterol transporter ABCA1 by 150% at the mRNA level, with ABCA1 protein levels being elevated by 80%. Cells treated with both TRAK2 and ABCA1 siRNA simultaneously displayed severely reduced cholesterol efflux relative to controls at levels comparable to treatment with ABCA1 siRNA alone, demonstrating that TRAK2 expression is dependent on ABCA1. Mass spectrometry studies further revealed an in vitro and in vivo correlation between TRAK2 expression and multiple lipid species, implicating a role for TRAK2 in broader lipid metabolism.

Conclusion: TRAK2 appears to regulate hepatic cholesterol efflux and therefore HDL-biogenesis through a pathway that is dependent on ABCA1. These studies validate the in vivo genetic and transcriptomic association between TRAK2 and HDL-cholesterol levels. The characterization of TRAK2 as a novel negative regulator of lipid metabolism has potential to progress the development of anti-atherosclerotic therapies.

STAINLESS STEEL-BOUND APAO-I AND RECONSTITUTED HIGH-DENSITY LIPOPROTEINS ARE ANTI-THROMBOTIC AND CAUSE ANTI-INFLAMMATORY EFFECTS IN HUMAN VASCULAR SMOOTH MUSCLE CELLS

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Background: Clinical efficacy of current endovascular stents is limited by thrombosis and inflammation-induced restenosis. Plasma activated coating (PAC) facilitates covalent binding of proteins to stainless steel (SS) surfaces in their bioactive state. High density lipoprotein (HDL) and its main apolipoprotein constituent apoA1 regulate key biological processes involved in restenosis and thrombosis, highlighting their potential for immobilization on PAC coated SS surfaces.

Objective: To determine if apoA1 or reconstructed HDL (rHDL) can be covalently bound to PAC-coated SS and, while retaining key biological properties that stand to improve stent biocompatibility.

Methods: Thrombosis was assessed on SS and PAC-coated SS by static assay and under flow using a modified changer loop. Smooth muscle cell (SMC) and endothelial cell (EC) attachment was quantified by crystal violet staining. Chemokine expression (CCL2, CCL5 and CXCL1) of TNF-α-stimulated SMCs grown on immobilized apoA1 and rHDL was measured by qPCR.

Results: The capacity of SS and PAC-coated SS to covalently retain apoA1 or rHDL was assessed using stringent SDS washing following protein incubation. On PAC-coated surfaces 58.1% of the original protein (before SDS washing) was retained, while on SS-only 16.2% was. Covalently retained apoA-I and rHDL on PAC-coated SS was 39.7 and 35.7 μg/cm², respectively. When exposed to heparinized whole blood, PAC-coated SS strikingly reduced thrombus formation relative to SS controls. However, for PAC+apoA-I and PAC+rHDL samples, thrombosis was completely absent, at 10, 30 and 60 min time points. Under flow, thrombus weight was reduced by 98%, 97% and 94% on PAC, PAC+apoA1 and PAC+rHDL, respectively, relative to SS (P<0.001). ApoA1 and HDL immobilized on PAC-coated SS reduced SMC attachment by 70% and 80%, respectively (P<0.001). Conversely, EC attachment was increased by 36% on PAC+apoA1 (P<0.05). Furthermore, SMCs plated on immobilized apoA1 and rHDL had significantly attenuated TNF-α-induced increases in CCL2 (40% and 47%), CCL5 (27% and 30%) and CXCL1 (36% and 36%) mRNA expression (P<0.05).
A LIPIDOMICS ASSESSMENT OF THE ANTI-OXIDATIVE CAPACITY OF HIGH-DENSITY LIPOPROTEINS

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Background: Oxidized low-density lipoprotein (LDL) may promote atherosclerosis. High-density lipoprotein (HDL) can protect LDL against oxidative damage by inactivating oxidized lipids as well as removal of oxidized lipids from LDL. Under conditions of chronic oxidative stress, HDL may become dysfunctional and so may contribute to the atherosclerotic process.

Aims: (1) To measure the formation of oxidized lipids in LDL during oxidation in the presence of native or oxidized HDL, and (2) to quantify the transfer of oxidized lipids from oxidized LDL to HDL.

Methods: Lipoproteins were isolated from pooled plasma of healthy volunteers (n=5) by sequential ultracentrifugation. Lipoprotein oxidation was catalysed using copper chloride. To achieve Aim 1, LDL was oxidized in the presence of native, mildly, or strongly pre-oxidized HDL. To achieve Aim 2, LDL was strongly oxidized and co-incubated with HDL followed by re-isolation. Oxidized phosphatidylcholine (oxPC) and oxidized cholesterol esters (oxCE) were determined by electrospray ionization liquid chromatography tandem mass spectrometry.

Results: oxPC and oxCE in LDL were reduced by 58% (n=3; P<0.01) and 38% (n=3; P<0.01), respectively, when oxidation was carried out in the presence of native HDL. However, pre-oxidation of HDL attenuated this process and only achieved 45% (n=3; P<0.01) and 12% (n=3; P<0.05) reduction in the formation of LDL-derived oxPC with mild and strong oxidative conditions, respectively. Co-incubation of oxidized LDL with HDL led to 71% and 28% reductions in LDL-derived oxPC and oxCE, respectively.

Conclusion: HDL was effective at reducing the formation of oxidized PC and oxidized CE residing on LDL. However, oxidation of HDL attenuated this process.

Conclusion: ApoA-I and rHDL can be covalently immobilized on to SS and exhibit anti-thrombotic and anti-inflammatory properties. This may represent a novel site-directed approach to increase stent patency.

RETARGETING GENETICALLY MODIFIED ADENOVIRUS 5 BY RGD4D INCLUSION IMPROVES VASCULAR TROPISM AND GENE TRANSFER

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Background: Cardiovascular disease is the second most common application for gene therapy clinical trials, which most frequently employ adenovirus serotype 5 (Ad5)-based vectors as delivery vehicles. Whilst gene therapy has great potential as a cardiovascular therapeutic strategy, intravascular delivery of Ad5 vectors are currently limited since they predominantly target the liver and interact with circulating proteins that reduce their efficacy. The development of more efficient vectors for vascular gene transfer is therefore warranted. We have shown that integration of amino acid mutations at key residues within hexon hypervariable regions (HVRs) generates an Ad5 vector (Ad5T*) that lacks hepatic tropism. Further modification of the virus by inclusion of targeting peptides may facilitate improved tropism and gene transfer efficiency to vascular cells.

Aim: To identify compatible locations for targeting peptide inclusion in genetically engineered adenovirus to enhance levels of vascular transduction and transgene expression.

Methods: For hexon modifications a βv3-integrin targeting peptide, RGD4C, was incorporated into either HVR 5 or 7 by an insertion (I) or replacement (R) strategy, while for fiber incorporation into either HVR 5 or 7 by an insertion (I) or replacement (R) strategy, while for fiber we inserted the peptide within the HI loop of the knob domain.

Results: RGD4C insertion within HVR7 proved incompatible with virus propagation, while insertion in HVR5 resulted in viral preparations with high viral particle to infectious particle ratios, suggesting packaging limitations when modifying certain regions of the virus capsid. In two βv3-integrin-positive cell lines, SKOV3 and A549, cell surface binding and viral transduction were significantly (P<0.001) increased for Ad5T*HVR7R RGD4C compared to control (Ad5T*). In cultures of human saphenous vein primary smooth muscle cells only Ad5T*HVR7R RGD4C increased cell surface binding (P<0.001), whereas transduction and transgene expression were significantly (P<0.001) increased with both Ad5T*HVR7R RGD4C and Ad5T*HI loop RGD4C compared to control (Ad5T*).

Conclusion: Both hexon HVR7 and fiber HI loop are viable locations for target peptide inclusion in Ad5T* which increases viral cell binding, uptake and transgene expression. These novel vectors with improved vascular transduction may provide vector platforms that enable the successful translation of human vascular gene transfer strategies.
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