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TOLERABILITY OF COMBINATION BLOOD PRESSURE LOWERING ACCORDING TO BLOOD PRESSURE LEVELS – AN ANALYSIS OF THE PROGRESS AND ADVANCE TRIALS

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Background: Combination blood-pressure lowering therapy provides greater blood pressure reduction than monotherapy and therefore greater reductions in major cardiovascular events. One concern is that combination therapy produces higher rates of adverse effects, particularly among patients with baseline systolic blood pressure below 140 mmHg. The ADVANCE and PROGRESS placebo-controlled trials of combination therapy recruited participants with a wide range of blood pressure levels and so provide a unique opportunity to investigate side-effects of combination therapy according to baseline blood pressure.

Aims: To measure the placebo-controlled effects of combination therapy on hypertension, treatment discontinuation and major renal outcomes, according to baseline blood pressure.

Methods: 14,684 participants allocated combination therapy or placebo were stratified into five groups by baseline systolic blood pressure (SBP) <120 mmHg, 120–129 mmHg, 130–139 mmHg, 140–159 mmHg, and ≥160 mmHg. Median follow-up in the randomized phase was 5.6 years, and discontinuation for hypertension and other causes.

Results: Discontinuation during the 4–6-week active-run-in phase due to hypotension/dizziness ranged from 3.6% in those with SBP <120 mmHg to 1.3% in those with SBP ≥160 mmHg. Median follow-up in the randomized phase was 5.6 years, and discontinuation for hypertension was higher with combination therapy compared to placebo in the <120 mmHg group (4.7% vs. 1.2%). However, for each subgroup with baseline SBP 120–129, 130–139 and 140–159 mmHg the absolute excess of discontinuation due to hypertension with combination therapy was 0.7%.

Conclusion: Compared to those with baseline SBP 140–159 mmHg, side-effects of dual combination blood-pressure lowering are essentially the same for people with SBP 130–139 mmHg. Median follow-up in the randomized phase was 5.6 years, and discontinuation for hypertension was higher with combination therapy compared to placebo in the <120 mmHg group (4.7% vs. 1.2%). However, for each subgroup with baseline SBP 120–129, 130–139 and 140–159 mmHg the absolute excess of discontinuation due to hypertension with combination therapy was 0.7%.

HUMAN AMMON NERVE CELL-DERIVED EXOSOMES IMPROVE STROKE OUTCOME IN MALE MICE

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Background: Recent findings by our Laboratory indicate that human amnion epithelial cells (hAECs), which are a placental stem cell, are neuroprotective following stroke. However, little is known about the factors that these cells release to elicit neuroprotection. Like most cell types, stem cells secrete extracellular microvesicles called exosomes, which are involved in cellular communication and thought to be the active component of stem cells. Aims: To test whether hAEC-derived exosomes exhibit similar neuroprotective effects as hAECs post-stroke.

Methods: Male mice (8–12 weeks old) were anaesthetised with intraperitoneal ketamine (80 mg/kg) and xylazine (10 mg/kg) and subjected to 30 min middle cerebral artery occlusion (mCAO). At 1 h following reperfusion, mice were injected intravenously with vehicle (saline, n=8); 108 hAECs (n=8) or 0.1 μg of hAEC-derived exosomes (n=6). After 24 h, functional outcomes and infarct volumes were assessed and immune cell infiltration and glial scarring were analysed via immunohistochemistry.

Results: Mice treated with either hAECs or exosomes were able to grip a wire for 53% and 45% longer compared to vehicle, respectively. Furthermore, the treated mice had a lower neurological deficit score compared to vehicle-treated mice. Infarct volume was reduced by 56% (P<0.05) and 65% (P<0.05) following hAEC and exosome administration, respectively. Consistent with hAEC treatment, exosomes prevented the increase in neutrophils and T cells in the ischemic hemisphere. Finally, exosomes abolished stroke-induced glial scar formation and hAECs reduced its size by 58%, although this was not statistically significant.

Conclusion: These data indicate that hAEC-derived exosomes provide similar neuroprotective effects as hAECs following ischemic stroke, thus demonstrating the potential of exosomes as a future stroke therapy.

DIETARY SALT INCREASES ARTERIAL STIFFNESS INDEPENDENT OF BLOOD PRESSURE AND LIFESTYLE FACTORS

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Background: Human studies show an association between high salt consumption and increased large artery stiffness, but are confounded by concomitant differences in blood pressure and altered lifestyle factors.

Aims: To examine the effects of a high-salt diet on aortic function independent of all other factors.

Methods: Sprague-Dawley rats were fed normal chow (control, 0.26% sodium chloride, n=6) or high-salt diet (HS, 8% sodium chloride, n=6) from weaning. A third group received a high-salt diet and an antihypertensive (HS+Tx, s.c. amloidipine 5 mg/kg/day, n=6). At 14–17 weeks, thoracic and abdominal aortic pressure (invasive solid-state catheters) was recorded under anesthesia over a mean arterial pressure (MAP) range of 60–150 mmHg (i.e. phenylephrine and sodium nitroprusside, 30 μg/kg/min). Aortic stiffness was assessed by pulse wave velocity (PWV) and thoracic and abdominal aortic pulse pressure amplification (PPA).

Results: Conscious systolic blood pressure (tail-cuff) was greater in HS rats (control 117±13 mmHg, HS 125±12 mmHg; P=0.004) and normalized by antihypertensive treatment (HS+Tx 112±13 mmHg, P=0.12). Higher blood pressure was associated with increased kidney mass (normalized to body weight, control 0.8±0.1%, HS 1.0±0.2%; P=0.002; HS+Tx 0.9±0.1%, P=0.17) without left ventricular hypertrophy (P=0.58). Food intake was similar amongst all groups but HS and HS+Tx rats drank more with correspondingly higher urine output. HS had higher PWV (across MAP range 60–150 mmHg; control 3.6±0.1 to 4.0±0.3 m/s; HS, 4.2±0.2 to 4.6±0.3 m/s; P<0.001 at each 5 mmHg MAP interval). Increased PWV was maintained with antihypertensive treatment (HS+Tx 3.9±0.1 to 4.8±0.1 m/s; P<0.001). PPA was greater in HS (across MAP range 60 to 150 mmHg; control 0.64±0.08 to 1.24±0.11, HS 0.89±0.02 to 1.54±0.04; P<0.001 at each 5 mmHg MAP interval) and with antihypertensive therapy (HS+Tx 0.79±0.05 to 1.39±0.03; P<0.001).

Conclusion: Under controlled blood pressure, a high-salt diet induced greater aortic stiffness and higher PPA, indicating salt effects on arterial stiffness are independent of blood pressure and other lifestyle factors.

FIRST EVIDENCE OF PULSATILE PRESSURE INTERACTION BETWEEN THE MACRO-VASCULATURE AND MICRO-VASCULATURE: PROOF-OF-CONCEPT BY ASSOCIATION WITH KIDNEY DYSFUNCTION AMONG PATIENTS WITH TYPE 2 DIABETES

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Background: It is widely thought that excess pulsatile pressure energy from increased stiffness of large central arteries (macro-vasculature) is transmitted to capillary networks (micro-vasculature) and causes end-organ damage (i.e. kidneys). However, this hypothesis has never been tested.

Aims: The aim of this study was to firstly determine the association between simultaneously measured macro-vascular and micro-vascular waveform features in people with increased macro-vascular stiffness (patients with type 2 diabetes; T2DM) compared with non-diabetic controls, and secondly to determine the association of waveform features with kidney function.

Methods: Among 13 T2DM patients (aged 68±6 years) and 15 controls (58±11 years) macrovascular function was measured by aortic stiffness and radial artery waveforms by tonometry. Forearm microvascular waveforms were simultaneously measured via low power laser Doppler flowmetry, with augmentation index (AIx) and augmented pressure (AP) derived on all waveforms. Kidney function was assessed by estimated glomerular filtration rate (eGFR).

Results: Aortic stiffness was higher among T2DM patients (9.3±2.5 vs. 7.5±1.4 m/s; P=0.046). There was an obvious pulsatile micro-vascular waveform, with qualitative features similar to radial waveforms, Macro-vascular AIx and AP were significantly related to micro-vascular waveforms AIx (r=0.428, P=0.005 and n=0.1245, P=0.004 respectively). Microvascular (but not macrovascular) AIx was associated with eGFR in T2DM (r=−0.632, P=0.037).
Conclusion: This is the first-in-human evidence of pulsatile pressure interaction between the macro-vasculature and micro-vasculature, and provides potential explanation for accelerated kidney dysfunction.

LONGITUDINAL CHANGES IN AORTIC RECURRENT FUNCTION INDEPENDENTLY PREDICT DECLINING RENAL FUNCTION AMONG HEALTHY INDIVIDUALS

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Background: Aortic recurrent function independently predicts end-organ damage in cross-sectional analyses. Longitudinal associations are more important regarding causation, but have never been examined.

Aims: To determine the longitudinal changes in aortic recurrent characteristics and the relationship with renal function in healthy individuals.

Methods: Aortic recurrent function (excess pressure integral [xpi] and aortic recurrent pressure, aortic stiffness, brachial and central blood pressure, and renal function estimated glomerular filtration rate [eGFR]) were recorded among 33 healthy individuals (age 57±9 years; 55% male) at baseline and after an average 3.3±0.3 years.

Results: Over the follow-up period there was no significant change in brachial BP (P>0.05), whereas there was a trend for xpi (P=0.061) and central BP (P=0.068) to increase. On the other hand, aortic stiffness and blood glucose increased significantly (P<0.05 for both). The change over time in xpi (but not aortic stiffness) was significantly related to the change in eGFR (r=-0.370, P=0.044) and this remained independent age, 24-hour systolic BP and body mass index (β=-0.031, P=0.045), but not blood glucose (β=0.031, P=0.053). There was no interaction between the change in glucose and change in xpi.

Conclusion: Aortic recurrent function, as determined by excess pressure, is independently associated with a decline in renal function among healthy people followed over 3 years. These novel findings indicate the need to determine the underlying physiological determinants of aortic recurrent function.

EPGENETIC CLUES TO THE BLOOD PRESSURE "LEGACY EFFECT" IN SPONTANEOUSLY HYPERTENSIVE RATS

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Background: In the spontaneously hypertensive rat (SHR) the "legacy effect" is the per -

Aims: To examine whether epigenetic changes might be associated with the "legacy effect" in the spontaneously hypertensive rat (SHR) the "legacy effect" is the persistent reduction in blood pressure (BP) and increased lifespan after short-term treatment with an angiotensin converting enzyme inhibitor (ACEi). However, the molecular machinery has never been examined.

Methods: 6-week-old male SHR were treated with the ACEi perindopril (1 mg/kg/d) (n=6) or vehicle (n=6) for 48 hours. Average global DNA methylation was quantified in renal cortices using the 5-mC ELISA Kit (Zymo Research, USA) which features a unique anti-

Results: Global DNA methylation was reduced in the renal cortices of animals treated with perindopril (P<0.05). And DNA (cytosine-5-)-

Conclusion: Global DNA methylation was reduced in the renal cortices of animals treated with perindopril, and DNA (cytosine-5-)-

Mct3 and Hdac1 >0.05).

<0.05, Figure 1). Acute treatment with perindopril did not significantly change the

Results: Global DNA methylation was reduced in the renal cortices of animals treated with perindopril (P<0.05). And DNA (cytosine-5-)-

Conclusion: Global DNA methylation was reduced in the renal cortices of animals treated with perindopril, and DNA (cytosine-5-)-

Figure 1. Global DNA methylation of vehicle treated (control) and treated (ACEi) groups. *P<0.05.

RELATIONSHIPS BETWEEN FATNESS AND FITNESS AND CARDIOMETABOLIC RISK FACTORS IN ADOLESCENTS FROM THE WESTERN AUSTRALIAN PREGNANCY (RAINE) COHORT STUDY

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Background: Independent effects of cardiorespiratory fitness and obesity on cardiovascular risk factors are well established in adults. However, their relative importance is uncertain, particularly during the crucial developmental stage of late adolescence.

Aims: To examine and compare the concurrent influences of cardiorespiratory fitness and fatness in relation to cardiometabolic risk factors in adolescents from the Western Australian Pregnancy (Raine) Cohort Study at age 17 years.

Methods: Fatness was determined from waist circumference. Cardiorespiratory fitness was estimated from heart rate recordings during sub-maximal cycle ergometry with adjustment for body weight. Fatness and fitness were assessed as continuous measures to avoid the use of arbitrary cut points and linear regression analyses were used.

Results: Fatness was positively associated with systolic blood pressure (P<0.001), triglycerides (P<0.001), low density lipoprotein-cholesterol (P=0.007) and high-sensitivity C-reactive protein (P<0.001). Fatness also increased the risk of being pre-hypertensive or hypertensive (P<0.001). There were no significant effects of fitness on any of these measures. A positive association between homeostatic model assessment of insulin resistance and fatness (P<0.001) was attenuated by fitness (P<0.001). Fatness was inversely associated with high density lipoprotein-cholesterol in both sexes (P<0.001), while fitness was positively associated with high density lipoprotein-cholesterol only in females (p=0.03). Fitness was inversely associated with diastolic blood pressure (P<0.001), whereas there was no association with fatness.

Conclusion: Fatness attenuated the adverse effects of fitness on few cardiometabolic risk factors. However, fatness had generally stronger associations with an adverse cardiometabolic profile in this population of 17-year-olds. Strategies to avoid excessive weight gain will need to focus on both eating patterns and physical activity in early childhood to minimize the risk of cardiovascular and metabolic disease in later life.

IMPORTANCE OF LYMPHOCYTES, ESTROGEN AND THE G PROTEIN COUPLED ESTROGEN RECEPTOR 1 IN ALDOSTERONE/SALT-INDUCED HYPERTENSION

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Background: The G protein-coupled estrogen receptor 1 (GPER), a membrane-localized estrogen receptor, may contribute to some of the effects of aldosterone. Aldosterone may not bind to GPER but it can promote interaction between the GPER and its classical target, the mineralocorticoid receptor G1, a GPER agonist, can exert T cell-mediated anti-inflammatory actions and has been reported to acutely lower blood pressure in normotensive male rats. We tested whether GPER activity might influence chronic pressor changes during aldosterone/salt-induced hypertension, and if those effects were sex-specific and immune cell-dependent.

Aims: To test the effects of G1 and G15 (GPER antagonist in two models of hypertension: 1) aldosterone/salt and 2) angiotensin II, and to examine the role of lymphocytes in those effects.

Methods: C57Bl6 mice (n=140) and RA1-G1-deficient mice (n=24) were treated with vehicle, aldosterone/salt (0.72 mg/kg/d s.c. plus 0.9% NaCl for drinking) or angiotensin II (0.7 mg/kg/d hypertension) (P<0.05). There were no significant changes in blood pressure was measured by tail-cuff. GPER expression was assessed by flow cytometry or immunofluorescence.

Results: In male C57Bl6 mice, aldosterone/salt caused a sustained increase in blood pressure of ~24 mmHg within 7 d and this increase was attenuated by ~50% in the presence of G1 (n=11–13, P<0.05). The antihypertensive effect of G1 was prevented by G15, whereas G15 alone did not alter hypertension caused by aldosterone/salt. By contrast, in male RA1-G1-deficient mice, aldosterone/salt had no effect on blood pressure (n=8, P<0.05). In female C57Bl6 mice, aldosterone/salt alone had no effect on blood pressure after 7 d, but co-administration of aldosterone/salt with G15 resulted in a profound increase of ~18 mmHg (n=6–8, P<0.05). By day 14, aldosterone/salt alone increased blood pressure in females to a similar level as aldosterone/salt + G15. Neither G1 nor G15 had any effect on angiotensin II-induced hypertension in male C57Bl6 mice (n=5–7). CD4+ T cells, CD8+ T cells, CD19+ B cells and F4/80+...
Background: Cardiac tissue engineering, particularly that utilizing autologous human stem cells, has the potential to produce constructs of cardiac tissue for surgical replacement of inefficient or damaged cardiac muscle or pacemaker tissue. Both pediatric and adult applications may be possible. Such constructs will also be useful as testing platforms for development of new drugs and pharmacological safety. We have established platforms to grow robust cardiac constructs with an integrated vasculature, constructs that grow and survive transplantation. Fully vascularized, robust beating cardiac tissue has been grown in pedicles constructed in polycarbonate chambers, and implanted in vivo in rats.

Aim: To grow integrated constructs of human cardiac tissue of mature ventricular phenotype from stem cells.

Methods: We have used both mesenchymal stem cells (MSC) and induced pluripotent stem (iPS) cells as sources of human cardiomycytes to grow cardiac constructs in vivo. Using the traditional embryonic body (EB) approach to generate beating, cardiomyocyte-like cells from iPSCs, we stimulated these differentiating cells electrically in bespoke chambers for short periods of up to 15 min. In a separate series of experiments, iPSCs were grown in monolayers, replated into the stimulating chambers for 4 days then stimulated continuously (1 ms, 200 mV/mm, 1 Hz) for 7 days. Finally other iPSC-derived cells grown in monolayers were replated into a fibrin matrix (>1.5 million cells per batch), then implanted into specially designed polycarbonate chambers incorporating miniaturized power packs and stimulating electrodes. These cell batches were wrapped around arterial and venous femoral vessels, implanted in vivo in immunocompromised rats, and stimulated for 4 h daily for 3 weeks, using a cage with pulsating orthogonal magnetic field, which generated charge-balanced electrical pulses in the chamber with the same parameters as those previously used in vitro. The tissues thus produced were examined after 3 weeks of stimulation in one leg, the tissues growing in the other with similar chambers that lacked stimulating electrodes, acting as controls.

Results: Stimulating IPS-derived cells electrically for prolonged periods (at 1 Hz up to 7 d) in vitro increased the maturation of derived cardiomyocytes towards an adult ventricular phenotype as assessed by several measures, including promoting their alignment with the electrical field. In vivo, disassociated cells did not survive the transplantation for 4 weeks, but transplanted human cell clumps grew into robust cardiac tissue in the constructs, characterized by troponin T striations interspersed with lecin-stained vessels. The modified bionic chambers described above, which enabled electrical stimulation of developing constructs in vivo, thus encouraged the development of contracting constructs in the chamber, incorporating human cardiac cells vascularized profusely by rat host vessels. In preliminary studies to date it was not possible to discern any difference resulting from 3 weeks of electrical stimulation in vivo. Further studies with more powerful and prolonged stimulating protocols are underway.

Conclusion: These tissue engineering approaches incorporating endogenous vascularization provide proof-of-principle for generation of substantial, transplantable cardiac tissue from human iPSCs. Electrical stimulation of sufficient power promotes maturation of the cardiomyocytes towards an adult ventricular phenotype, a goal that has eluded many in the field to date.

VITAMIN D SUPPLEMENTATION REDUCES BRAIN INJURY AND INFLAMMATION FOLLOWING ISCHEMIC STROKE
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Background: Following ischemic stroke, inflammation is a major contributor to secondary brain injury and further tissue infarction. Beyond its well-characterized role in calcium metabolism, the active form of vitamin D, 1,25-dihydroxyvitamin D3 (1,25-VitD3), has been shown to elicit anti-inflammatory actions. Given the contributing role of the immune system in acute post-stroke brain injury, we hypothesized that vitamin D3 supplementation may reduce brain injury in association with reduced inflammation following stroke.

Aims: To determine whether supplementation of 1,25-VitD3 reduces inflammatory brain injury after ischemic stroke in mice.

Methods: Male C57Bl/6j mice (aged 7–10 weeks) were randomly assigned either 1,25-VitD3 (n=32; 100 ng/kg/d for 7 days) or vehicle (n=30; mixture of ddH2O, propylene glycol and ethanol) commencing 5 d prior to stroke. Stroke was induced via middle cerebral artery occlusion for 1 h followed by 23 h reperfusion. Twenty-four hours after stroke induction, hanging grip and parallel rod tests were used to assess grip strength and locomotor activity, respectively. In addition, infarct volume was assessed by thionin staining and cerebral inflammation was evaluated using real-time PCR and immunohistochemistry.

Results: Supplementation with 1,25-VitD3 reduced cerebral infarct volume by 50% compared to vehicle (18±3 mm3 versus 36±6 mm3, respectively; P<0.05). However, at this early time-point there were no differences in functional outcomes, with hanging grip time and total time mobile being similar in 1,25-VitD3- and vehicle-supplemented groups. Expression of key pro-inflammatory cytokines, IL-6, IL-1β and IL-10, was decreased in brains of mice that received 1,25-VitD3 versus vehicle (n=9–12, P<0.05). Expression of the T regulatory cell marker, FOXP3, was further elevated in mice supplemented with 1,25-VitD3 (n=11 per group; P<0.05). Immunosuppression was indicated by the ratio of neutrophils and T cells infiltrating the ischemic hemisphere were similar in 1,25-VitD3- and vehicle-supplemented groups (6–7:1). Ongoing experiments are assessing other immune cell types, and whether pre-existing vitamin D deficiency predisposes to a worse stroke outcome.

Conclusion: These data indicate that administration of exogenous vitamin D to vitamin D-replete mice can attenuate infarct development and exert anti-inflammatory actions. This may represent a direction for acute stroke therapy.

POST HOC ANALYSIS OF THE EFFECTIVENESS OF BLOOD PRESSURE-LOWERING DRUG TREATMENT BY LEVELS OF ABSOLUTE RISK IN THE ANBP STUDY
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Background: Cardiovascular disease (CVD) continues to represent a considerable burden on the health care system. International guidelines for the primary prevention of CVD recommend drug treatment for elevated blood pressure (BP) based on BP thresholds with due deference to underlying absolute CVD risk. Guidelines in Australia and New Zealand give pre-eminence to risk stratification using risk calculators to determine BP-lowering drug treatment thresholds. However clinicians are concerned that the average risk approach compared with a BP threshold may be an inferior strategy.

Aims: To examine if BP-lowering treatment based on baseline CVD risk would have superior outcomes compared with a simple BP threshold for those with "mildly" elevated BP.

Methods: We conducted a post hoc subgroup analysis of the Australian National Blood Pressure study (ANBP). The ANBP study was a randomized placebo controlled trial, in which participants were recruited from the community with "mildly" elevated diastolic BP between 1973 and 1979. In the present study, we involved participants aged 35 to 69 years. All analyses were based on the "intention to treat." The Cox proportional hazard model was used to estimate the hazard ratios (HRs) and corresponding 95% confidence intervals for participants classified by tertile of Framingham risk scores.

Results: Participants had an average 5-year CVD risk in the intermediate range (10.5±6.5) with moderately elevated BP (159±103 mmHg) and were middle-aged (52±8 years). We identified no significant effect of BP lowering drug treatment on major CVD events with a HR 0.83(95%CI 0.65–1.07) or all-cause mortality with a HR 0.75(95%CI 0.45–1.26) and a borderline significant effect of stroke with a HR 0.55(95%CI 0.31–1.00). In subgroup analyses, the relative and absolute effects did not significantly differ across the CVD risk groups. In terms of absolute benefit, BP-lowering drug treatment significantly reduced the number of events in the high-risk tertile with respect to any event with a number needed to treat (NNT) of 19 (95% CI 11–78), death from any cause with a NNT of 49 (95% CI 26–330) and major CVD event with a NNT of 23 (95% CI 12–164).

Conclusion: BP-lowering drug treatment produced non-significant effects in the overall study population. Our analysis confirms that the benefit of treatment was substantial only in the high-risk tertile, reaffirming the rationale of treating elevated BP in the setting of all risk factors rather than in isolation.

CCL18 AS A MEDIATOR OF THE PRO-FIBROTIC ACTIONS OF M2 MACROPHAGES IN THE VESSEL WALL DURING HYPERTENSION

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Background: M2 macrophages contribute to vascular fibrosis and stiffening in hypertension. A potential mediator of these actions is the macrophage-derived, pro-fibrotic chemokine, CCL18, which signals via its cognate receptor, CCR8. Little is known about the role of CCL18 in cardiovascular disease. In addition, the localization and expression of CCR8 in the vascular wall has not been investigated.

Aims: To determine if angiotensin II augments CCL18 production from human primary M2 macrophages, identify cardiovascular targets of CCL18 and investigate the ability of CCL18 to promote fibrosis.
GA
GG
AA
mass (5, 16]. The SNP allele is associated with LVH in patients without diabetes. Studies are needed to characterize the functional importance of these results, and to deter-
influences myocardial KLF15 of age, gender, BMI, systolic blood pressure and hypertension.
allele compared to those without LVH and the rs9838915 A in 35% of patients. Over a median follow up of 5.6 years, there were 22 (7%) heart failure
KLF15 rs6918698 and LV mass. LVH was present =0.004) wall thickness. There were no
P = 0.001) adjustment for age, gender, BMI and hypertension, and
P = 0.003) and after (vi) aortic-to-brachial and brachial-to-radial SBP-amplification (n=18). Compared with the first three phenotypes, patients with no SBP-amplification had elevated aortic SBP (144±23 mmHg vs. 125±19; 130±23 and 135±11 mmHg, respectively; P=0.019) that was significantly underestimated by each brachial cuff BP device (12±9.21 mmHg; P=0.048 (A) and –8.4±6.5 mmHg; P=0.002 (B), despite no differences in clinical characteristics or cuff BP between phenotypes (P=0.05 for all).
Conclusion: These are the first data to describe distinctive central-to-peripheral SBP-
amplification phenotypes, and includes discovery of a phenotype in which cardiovascular risk is likely to be elevated by because of significantly increased aortic SBP that is not detected by conventional cuff BP methods.

GENETIC VARIATION IN KRUPPEL-LIKE FACTOR 15 INFLUENCES MYOCARDIAL MASS IN PATIENTS WITH TYPE 2 DIABETES AND IS ASSOCIATED WITH HEART FAILURE HOSPITALIZATION
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Background: Left ventricular (LV) hypertrophy (LHV) is prevalent in type 2 diabetes and associated with adverse cardiovascular (CV) outcomes including heart failure. The trans-
sectional factor Kruppel-like factor 15 (KLF15) is expressed in the heart and acts as a repressor of cardiac hypertrophy. Aims: To investigate whether KLF15 gene variants are associated with increased LV mass and heart failure hospitalization in patients with type 2 diabetes. Methods: We recruited 346 asymptomatic patients with type 2 diabetes for transthoracic echocardiography (Melbourne Diabetes Heart Cohort). Patients with valvular dysfunction/ replacement/repair (n = 28) were excluded. No patient had a previous history of heart fail-
ure. Two common KLF15 tagging single nucleotide polymorphisms (SNPs) were genotyped (rs9838915, rs9769225) in 318 patients. LVH was defined as LV mass (indexed to body surface area) of >115 g/m² in men and >95 g/m² in women. Results: The mean age (±SD) was 64±12 years (64% male). BMI was 32±6 kg/m², hyper-
tension was present in 79% and median diabetes duration was 10 years [25, 75th quartile 5, 16]. The KLF15 SNP rs9838915 A allele was associated in a dominant manner with LV mass (GA-AA genotype vs. GG homozygote: 105.8±28.3 vs. 95.9±25.5 g/m² before (P = 0.003) and after (P = 0.001) adjustment for age, gender, BMI and hypertension, and with adjusted septal (P=0.001) and posterior (P=0.004) wall thickness. There were no significant associations between the KLF15 SNP rs9769225 and LV mass. LVH was present in 35% of patients. Over a median follow up of 5.6 years, there were 22 (7%) heart failure hospitalizations.

Conclusion: We report for the first time that genetic variation in KLF15 influences myocardial mass in patients with type 2 diabetes and is associated with heart failure hospitalization. Studies need to characterize the functional importance of these results, and to deter-
mine if the KLF15 SNP rs9838915 A allele is associated with LVH in patients without diabetes.

DISCOVERY OF A NEW BLOOD PRESSURE PHENOTYPe FROM INTRA-ARTERIAL CENTRAL-TO-PERIPHERAL RECORDINGS: IMPLICATIONS FOR CUFF BLOOD PRESSURE ACCURACY AND CARDIOVASCULAR RISK ASSESSMENT
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Background: Individual variability in central-to-peripheral systolic blood pressure (SBP) amplification might make it difficult for brachial cuff blood pressure (BP) to accurately reflect intra-arterial BP, but this has never been determined.

Aims: To characterize SBP-amplification phenotypes and examine the association of these with cuff BP accuracy.

Methods: Following coronary angiography, intra-arterial BP was measured at the ascending aorta, brachial and radial arteries in 99 patients (aged 61.9±10.0 years; 68% male). Cuff BP was measured using two separate oscillometric devices at the following times: (A) bilaterally before catheterization (AoD UA-767) and (B) simultaneously with intra-arterial brachial and radial pressure. SBP-amplification was defined by ≥ 5 mmHg SBP increase between the aorto-brachial and brachial-to-radial arteries.

Results: Average aorto-brachial and brachial-to-radial SBP-amplification were 8.1±9.2 mmHg and 6.4±10.1 mmHg respectively. However, four distinct SBP-amplification pheno-
types were observed: (i) both aortic-to-brachial and brachial-to-radial SBP-amplification (n=29); (ii) only aortic-to-brachial SBP-amplification (n=30); (iii) only brachial-to-radial SBP-amplification (n=25); (iv) no aortic-to-brachial or brachial-to-radial SBP-amplification (n=18). Compared with the first three phenotypes, patients with no SBP-amplification had elevated aortic SBP (144±23 mmHg vs. 125±19; 130±23 and 135±11 mmHg, respectively; P=0.019) that was significantly underestimated by each brachial cuff BP device (12±9.21 mmHg; P=0.048 (A) and –8.4±6.5 mmHg; P=0.002 (B), despite no differences in clinical characteristics or cuff BP between phenotypes (P=0.05 for all).

Conclusion: These are the first data to describe distinctive central-to-peripheral SBP-
amplification phenotypes, and includes discovery of a phenotype in which cardiovascular risk is likely to be elevated by because of significantly increased aortic SBP that is not detected by conventional cuff BP methods.

RENAI DENERVATION PREVENTS PROGRESSION OF HYPERTENSION AND CHANGES TO BAROREFLEX IN A RABBIT MODEL OF CHRONIC KIDNEY DISEASE
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Background: Hypertension associated with chronic kidney disease (CKD) rapidly pro-
gresses to become treatment-resistant but the benefits of renal denervation (RDN) in this disease are unknown. A rabbit model of moderate CKD, induced by 11/12 nephrectomy and characterized by rapid and sustained elevation of plasma creatinine and hypertension, allows for determination of the effects of RDN and whether its efficacy improves with time.

Aims: To determine the effects of RDN on CKD-induced changes to mean arterial pressure (MAP), renal sympathetic nerve activity (RSNA), baroreflex function and renal function and whether they are altered by time after RDN.

Methods: Rats underwent RDN of the left renal nerve 2 weeks after CKD was induced by lesion of the left kidney and right nephrectomy. In sham rabbits, both kidneys were denervated. MAP, RSNA recorded from the left renal nerve and baroreflexes were examined 2 and 4 weeks after RDN.

Results: Two weeks after induction of CKD, creatinine increased by 59% and remained elevated by 41% over the following 4 weeks. MAP had increased by 14% to 77±1 mmHg after CKD and continued to rise from this level by +8% and +13% after 2 and 4 weeks in sham RDN rabbits, while creatinine did not change. However, in rabbits which under-
went RDN there was no further increase in MAP (P=0.001 after 4 weeks RDN). RSNA was 33% lower 2–3 weeks following RDN than after sham RDN (P<0.05) and the hypertensive response to pentolinium was reduced by 50%. CKD shifted the RSNA baroreflex towards the higher MAP and this was reversed after 2 weeks of RDN with marked reductions in gain (38%) and range (42%). There was no further change to the baroreflex after 4 weeks RDN. Neither CPR (14±2 mL/min in controls), which was 45% lower in CKD rabbits, nor creatinine levels, were altered by 4 weeks of RDN.

Conclusion: RDN is effective for at least 4 weeks in ameliorating the hypertension in this model of CKD. Sympathetic activation and changes to the RSNA baroreflex were also reversed without altering renal function. Our results suggest that RDN may be an effective treatment for moderate CKD.

EXAGGERATED EXERCISE BLOOD PRESSURE IS ASSOCIATED WITH RAISED LEFT-VENTRICULAR MASS AND AORTIC STIFFNESS IN ADOLESCENCE: A cross-sectional analysis of the Avon Longitudinal Study of Parents and Children (ALSPAC)
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Background: Dynamic exercise results in increased systolic blood pressure (BP), irrespective of resting BP; some individuals may experience an exaggerated rise in systolic BP with exercise, which in adulthood is associated with risk of developing hypertension, cardiovascular (CV) morbidity and mortality. It is unknown if exercise BP is associated with adverse CV risk during adolescence.

Aims: In a large group of adolescents, we aimed to determine if exercise systolic BP was associated with hypertension-related CV risk markers (left-ventricular mass (LVM) and
Aortic stiffness independent of resting (office) BP status, with further consideration of the possible confounding effect of body composition.

Methods: A total of 4,036 adolescents (mean age 17.8±0.4 years, 45% male), part of a UK population-based birth cohort study, completed a sub-maximal step-test with BP measurement post-exercise. Sub-samples underwent assessment of echocardiographic LVM (n=2,067), aortic pulse wave velocity (aPWV; n=3,582) and body composition by dual-energy x-ray absorptiometry (DXA; n=3,875). Resting BP status was classified as raised if systolic and/or diastolic BP was in the ≥95th percentile.

Results: Each 5 mmHg increase in post-exercise systolic BP was associated with a 0.31 g/cm² (95% CI 0.22, 0.40) greater LVM index, and 0.03 m/s (95% CI 0.02, 0.03) increased aPWV after adjustment for age, sex and resting BP status (P<0.05). Further adjustment for DXA-measured total body fat and lean mass attenuated the relationship with LVM to 0.14 g/cm² (95% CI 0.05, 0.22; P<0.001) and aPWV to 0.02 m/s (95% CI 0.02, 0.03) per 5 mmHg of post-exercise systolic BP (P<0.001).

Conclusion: In adolescents, exercise systolic BP is associated with hypertension-related CV risk markers that are prognostic in adults independent of resting (office) BP status. Adjustment for body composition attenuates, but does not abolish, these associations. Given the clinical relevance of exaggerated exercise BP in adulthood, these results may have implications for CV risk in later life.

VARIABILITY IN CENTRAL-TO-PERIPHERAL GRADIENTS OF MEAN ARTERIAL PRESSURE AND DIASTOLIC BLOOD PRESSURE WITHIN THE HUMAN LARGE ARTERIES: RELEVANCE TO ARTERIAL PHYSIOLOGY AND ESTIMATED CENTRAL BLOOD PRESSURE


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Background: In order to aid blood flow, the gradient of mean arterial pressure (MAP) and diastolic blood pressure (DBP) is thought to decline approximately 1–3 mmHg through the large arteries. The magnitude of this gradient is also of potential importance for accurate waveform calibration and non-invasive estimation of central BP. However, there are few data determining the individual variability of MAP and DBP gradients from central to peripheral large arteries.

Aims: To determine the magnitude and variability in MAP and DBP gradients from the aorta to the brachial and radial arteries.

Methods: A total of 75 patients (mean age 62±11 years) undergoing angiography had intra-arterial BP measured via fluid-filled catheter in the ascending aorta, brachial and radial arteries by sequential pull-back. MAP was calculated by integration of ensemble averaged waveforms, and DBP from the foot of the waveforms at each intra-arterial site.

Results: On average, MAP and DBP gradients decreased over the aortic-brachial (MAP –1.4±1.0 mmHg; DBP –2.5±2.3 mmHg), brachial-radial (MAP –2.4±1.1 mmHg; DBP –2.0±3.1 mmHg) and aortic-radial (MAP –3.8±4.4 mmHg; DBP –4.5±3.6 mmHg) arterial segments. The magnitude of the aortic-radial MAP (range –14.9 to 6.8 mmHg) and DBP (range –13.1 to 2.6 mmHg) gradient was, however, highly variable, and included reversal of the MAP and DBP gradient (increase) among 23% and 12% of patients, respectively.

Conclusion: Although MAP and DBP gradients reduce on average from central to peripheral large arteries, the magnitude of this gradient is variable. These new observations are highly relevant to understanding arterial hemodynamic pathophysiology and accurate non-invasive estimation of central BP.

VARIABILITY IN BRACHIAL-TO-RADIAL SYSTOLIC BLOOD PRESSURE MEASUREMENT: EFFECTS ON THE ACCURACY OF CENTRAL SYSTOLIC BLOOD PRESSURE ESTIMATED FROM THE RADIAL ARTERY: AN INTRA-ARTERIAL STUDY


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Background: Central systolic blood pressure (SBP) can be non-invasively estimated from radial pressure waveforms. Calibration of the radial waveform using brachial BP and DBP is advocated on the assumption of negligible brachial-to-radial SBP amplification. However, recent evidence suggests there may be significant variability in brachial-to-radial SBP amplification, and this could affect accuracy of central BP estimation, although has never been examined.

Aims: To determine the level of brachial-to-radial SBP amplification and the consequent effect on the accuracy of estimated central SBP.

Methods: Intra-arterial BP was measured via sequential catheter pull-back at the ascending aorta, brachial and radial arteries among 77 patients (mean age 62±11 years) undergoing diagnostic coronary angiography. Accuracy of estimated central SBP was assessed by comparison of intra-arterial central SBP with estimated central SBP (SyphgoMonCor) derived by calibrating the radial pressure waveform with either brachial or radial SBP and diastolic DBP (DBP).

Results: SBP increased stepwise from the aorta to the brachial and radial arteries (128.6±18.8, 137.9±18.6, 140.2±21.5 mmHg, respectively; P<0.001). Calibration of the radial pressure waveform with brachial SBP and DBP resulted in a significant underestimation of central SBP (–8.7±9.3 mmHg, P<0.001). Recalibration with radial SBP and DBP significantly (P<0.001) improved the accuracy of estimated central SBP (–5.2±10.4, P<0.001).

Conclusion: There is significant variability in brachial-to-radial SBP amplification and this affects the accuracy of estimated central SBP when radial pressure waveforms are calibrated with brachial SBP and DBP. Thus, brachial SBP and DBP should not be used to calibrate radial pressure waveforms in the estimation of central BP.

PRESSURE-INDUCED T CELL INFILTRATION IS CAVEOLIN-1 DEPENDENT


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Background: A common cause of cardiac hypertrophy is high blood pressure that we have previously shown to induce changes in endothelial phenotype via a caveolin-1 (cav-1) dependent mechanism. A role for the adaptive immune system has been established in the onset and progression of hypertrophy. Specifically, T cell infiltration is thought to play a pivotal role.

Aims: To investigate the contribution of pressure to the infiltration of T cells in both in vitro and in vivo settings and its subsequent effects on cardiovascular pathology.

Methods: Supernatants collected from cav-1 scrambled (SCR) or knockdown (KD) mouse endothelial cells that were either untreated, treated with the known migration stimulus CCL21 (100 ng/mL) or TNF-α (20 ng/mL) that were under 0 or 120 mmHg for 24 hours, were examined for their ability to induce T cell migration in a dual chamber migratory assay system. The number and phenotype of migrated T cells was assessed via flow cytometric analysis. In addition, we examined whether T cell migration into the heart was altered in the 4 week angiotensin II (Ang II; 490 ng/min/kg) induced hypertensive model in wild-type (C57BL/6) and cav-1 –/– mice. Systolic blood pressure (SBP) and cardiac hypertrophy were also assessed.

Results: Compared to untreated, unpressurized cells (8,020±2,772 SD, n=4), T cell migration was induced by supematant from C572-21 treated SCR cells (27,612±3,117, n=7; P<0.001), TNF-α-treated cells (25,938±5,173, n=10; P<0.001) and pressurized SCR cells (32,913±1,458, n=6; P<0.01). However, a significant increase in T cell migration was observed only in the TNF-α and CCL21-treated cav-1 KD cells (P<0.001), but not in the pressurized setting (2,232±749, n=12) relative to the untreated cav-1 KD cells (4,405±1,857, n=4; P<0.05). Of note, approximately 50% of migrated T cells in all treatment groups were of the T helper subtype. Although an increase in SBP was observed in both Ang II-treated wild-type and cav-1 –/– mice, increases in T cell infiltration in the heart were observed only in Ang II-treated wild-type mice, but not in Ang II-treated cav-1 –/– mice relative to their controls. This was accompanied by Ang II-induced cardiac hypertrophy in the Ang II-treated wild-type, but not the Ang II-treated cav-1 –/– mice compared to the untreated wild-type and cav-1 –/– mice, respectively.

Conclusion: We found that elevated pressure induces T cell migration and infiltration via a cav-1 dependent mechanism. Since cardiac hypertrophy was not exacerbated despite the increase in SBP in AT-treated cav-1 –/– mice, we postulate that cardiac T cell infiltration may play a role, potentially via a fibrotic process.

PREGNATAL HYPOXIA AND A POSTNATAL HIGH SALT DIET PREDISPOSES MOUSE OFFSPRING TO CARDIOVASCULAR AND RENAL IMPAIRMENTS IN ADULTHOOD


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Background: In Australia, chronic kidney disease and cardiovascular disease are prominent public health issues. Both are linked to abnormal kidney development, notably reduced nephron number, which may result from interference with early renal development.

Aims: We evaluated the impact of prenatal hypoxia on renal and cardiovascular development and function in the mouse, and whether high salt intake could exacerbate functional impairments.

Methods: Pregnant CDF1 mice were housed in a hypoxic chamber (12.0% O2) or control (21.0% O2) environment from embryonic day 14.5 to 18.5 (birth). Offspring were controlled to control (NaCl) or high-salt (NaCl) diets from 10 weeks to 12 months of age. Renal function was examined via 24 h metabolic cages and blood pressure was measured by radiotelemetry at 12 months of age. Mesenteric arteries were collected for pressurized in
vitro microscopy studies. Kidneys were used to determine nephron number by unbiased stereology. Hearts and kidneys from 12-month-old offspring were evaluated by an expert pathologist.

Results: Male hypoxia-exposed offspring presented with elevated urinary albumin excretion at 12 months of age. This was associated with a 25% reduction in nephron endowment, significant glomerular hypertrophy and glomerulosclerosis compared to male control offspring. These histopathological changes were exacerbated by the high-salt diet. In contrast, female hypoxia-exposed offspring had normal nephron endowment and no overt signs of renal impairment or histopathology. Male and female hypoxia-exposed offspring both presented with an approximately 14 mmHg increase in mean arterial pressure and mild vascular endothelial dysfunction. Consumption of a high-salt diet in both sexes led to marked mesenteric vascular stiffening and cardiac fibrosis in hypoxia-exposed offspring.

Conclusion: Prenatal hypoxia perturbed kidney development, impaired renal function and increased susceptibility to salt-induced renal injury in male offspring. Both sexes developed signs of cardiovascular disease in adulthood. This suggests that female offspring are afforded some renoprotection from hypoxia in utero. However, this protection does not extend to the cardiovascular system.

PLASMA POTASSIUM NEGATIVELY EFFECTS ABUNDANCE OF THE THAZIDE SENSITIVE SODIUM-CHLORIDE COTRANSPORTER IN HUMANS

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Background: The thiazide sensitive sodium-chloride cotransporter (NCC) is important for sodium reabsorption and blood pressure. With no-lyseine-kinase 4 (WNK4) is now thought to be an important regulator of NCC, and is mutated in some cases of Gordon’s syndrome, causing hypertension and hyperkalemia. We have previously demonstrated that WNK4 and NCC in human urinary exosomes appears to be sensitive to mineralocorticoids, but recent animal studies suggest that potassium is a dominant regulator of NCC, possibly via regulation of WNK4.

Aims: To test the effect of potassium on regulation of NCC and WNK4.

Methods: We isolated urinary exosomes from 20 subjects (10 with primary aldosteronism and 6 cured after adrenalectomy for aldosterone-producing adenoma), before fludrocortisone suppression testing and again after 3 days of fludrocortisone administration, and quantified abundance of NCC, phosphorylated NCC (pNCC) and WNK4 by Western blot.

Results: The patients with cured primary aldosteronism had lower aldosterone (144 pmol/L vs. 643 pmol/L; P=0.001) and a higher potassium (4.7 mmol/L vs. 3.6 mmol/L; P<0.001) compared to those with primary aldosteronism. NCC was > 4 fold, pNCC was > 5.5 fold, and WNK4 was > 6 fold higher in patients with primary aldosteronism compared to those who had been cured (P=0.05 for all). There were very strong negative correlations at baseline between plasma potassium and WNK4 (R2=0.57; P<0.001), NCC (R2=0.66; P<0.001) and pNCC (R2=0.43; P<0.01) compared to those with primary aldosteronism. There were weaker positive associations between plasma aldosterone and NCC (R2=0.34; P<0.01) and WNK4 (R2=0.24; P=0.03). After 3 days of fludrocortisone administration, however, there was no apparent relationship between potassium and abundance of NCC, WNK4 or pNCC.

Conclusion: NCC and its phosphorylated form pNCC are upregulated in primary aldosteronism, along with the regulatory kinase WNK4. Plasma potassium is closely related to the abundance of WNK4, NCC and pNCC, suggesting that potassium plays a role in NCC regulation, but might be over-ridden by mineralocorticoid stimulation in some circumstances.

HRPBCA Poster Presentations

EFFECT OF MOXONIDINE ON THE ALDOSTERONE/RENIN RATIO CALCULATED BY BOTH PLASMA RENIN ACTIVITY AND DIRECT RENIN CONCENTRATION IN HEALTHY MALE VOLUNTEERS

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Background: Plasma aldosterone/renin ratio (ARR) is the most popular screening test for primary aldosteronism (PA). Both estrogen and progesterone affect aldosterone and renin levels, but the effects of combined hormonal replacement therapy (HRT) on ARR have not been studied.

Aims: To examine the effects of combined hormonal replacement therapy (HRT) on ARR, measuring renin as both direct renin concentration (DRC) and plasma renin activity (PRA).

Methods: 15 norhormone, healthy premenopausal women underwent measurement (seated, midmorning) of plasma aldosterone, DRC, PRA, electrolytes and creatinine and urinary aldosterone, cortisol, electrolytes and creatinine at baseline and after 2 and 6 week’s treatment with combined HRT (conjugated oestrogen 0.625 mg and medroxyprogesterone 2.5 mg daily).

Results: Treatment with combined HRT was associated with significant increases in aldosterone (baseline median [range] 150 [85–600], 2 weeks 230 [129–790], 6 weeks 434 [200–1200] pmol/L; P<0.001 [Friedman test]) and decreases in DRC (21 [10–31], 21 [10–39], 14 [8.0–30] mU/L; P<0.01) leading to increases in ARR calculated by DRC (7.8 [3.6–34.8], 11.4 [5.4–48.5], 10.5 [9.0–90.2]; P<0.001). The ARR calculated by DRC exceeded the cut off value (70) in three patients after 6 weeks. There were no significant changes in ARR calculated by PRA (79 [26–184], 91 [23–166], 88 [50–230]; P=0.282), plasma electrolytes and creatinine, and all urinary measurements.

Conclusion: The combined oral HRT used in this study is capable of significantly increasing ARR with a risk of false positive results during screening for PA, but only if DRC (and not PRA) is used to calculate the ratio.

FLAVONOID-RICH APPLE IMPROVES ENDOTHELIAL FUNCTION IN INDIVIDUALS AT RISK FOR CARDIOVASCULAR DISEASE

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Background: Studies show an inverse association between apple intake and coronary mortality. The health benefits of apples have been attributed to their flavonoid content, which is considerably higher in the peel compared to the flesh. Endothelial dysfunction occurs before vascular disease is detected and is an independent predictor of cardiovascular risk. Our previous research has shown an improvement in endothelial function following apple consumption. To determine if the benefits of apples are due to their flavonoid-rich skin, we gave volunteers apples with skin or apple flesh-only as a control.

Aims: To determine if acute and chronic consumption of flavonoid-rich apple would improve endothelial function and BP in human volunteers with at least one risk factor for CVD.

Methods: We conducted a randomized, controlled, crossover trial with 30 men and women with one or more of the following: 120 < systolic BP < 160, 5.6 < blood glucose < 6.5, < total cholesterol < 8 or central obesity (men > 94 cm; women > 80cm). We assessed acute and chronic changes in BP, endothelial function, arterial stiffness, and levels of plasma flavonoid metabolites, cholesterol, glucose, nitrate and nitrite. Differences between interventions were assessed by mixed models ANOVA with adjustments for baseline.

Results: We observed significant improvements in endothelial function 2 hours after acute ingestion (0.7%, P<0.0001) and after 4 weeks chronic ingestion (0.58%, P<0.0001) of the apple skin intervention. We saw a significant increase in plasma quercetin metabolites after the acute and chronic apple with skin intervention. We saw no significant changes in other measurements.
Background: Inter-arm differences in brachial systolic blood pressure (SBP) may be real and cardiovascular risk predictive of outcome. If real, measurement of either arm should result in result in similar calculated central, aortic SBP, there being only one aortic blood pressure (ABP) measurement. We assessed whether inter-arm differences in ABP develop and whether these differences are correlated with aortic geometry.

Methods: Brachial SBP was measured simultaneously in both arms 4 times (alternating left-right) over a 4-week study period. Participants were aged 95-124 years (n=20). The correlation between brachial SBP and aortic SBP was determined using a non-invasive/magnetic resonance imaging (MRI) protocol.

Results: There were no significant correlations between inter-arm differences in brachial SBP and aortic SBP. There was no evidence of aortic geometry influencing brachial SBP.

Conclusion: There were no significant differences in brachial SBP between the two arms, and no evidence of aortic geometry influencing brachial SBP.

Risk Factors Associated with Hypertensive Disorders of Pregnancy Within an Urban Indigenous Population in Southwestern Sydney

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Aims: To explore the current salient risk factors for hypertensive disorders of pregnancy (HDP) in Australia’s Indigenous women. The aetiology of which remains to be fully elucidated. We explored the risk factors associated with HDP for a cohort of urban Indigenous women in southwestern Sydney, Australia.

Methods: A randomized controlled crossover study was performed in 15 healthy volunteers, which each completed 5 visits with a minimum washout period of 1 week in between testing days. Participants received each of the 5 interventions, in a random order: (i) 0 mg, (ii) 50 mg, (iii) 100 mg, (iv) 200 mg or (v) 400 mg quercetin-3-O-glucoside. Endothelial function and blood pressure were assessed before and after 60 min post-intervention. A blood sample was taken before and after 90 min for analysis of plasma nitrate and nitrite as markers of NO production, as well as plasma quercetin metabolites.

Results: A randomized controlled crossover study was performed in 15 healthy volunteers, each of whom completed 5 visits with a minimum washout period of 1 week in between testing days. Participants received each of the 5 interventions, in a random order: (i) 0 mg, (ii) 50 mg, (iii) 100 mg, (iv) 200 mg or (v) 400 mg quercetin-3-O-glucoside. Endothelial function and blood pressure were assessed before and after 60 min post-intervention. A blood sample was taken before and after 90 min for analysis of plasma nitrate and nitrite as markers of NO production, as well as plasma quercetin metabolites.

Conclusion: There were no significant correlations between inter-arm differences in brachial SBP and aortic SBP. There was no evidence of aortic geometry influencing brachial SBP.
Background: Minor alleles of multiple genetic variants of FOXO3 are associated with longevity, principally via protection against CAD mortality. Telomere shortening with age may affect lifespan. Telomere length is reduced in hypertension. Genetic variation in FOXO3 is associated with blood pressure and hypertension.

Aims: To assess the effect of longevity-associated FOXO3 and APOE alleles on telomere attrition with age.

Methods: The study involved 127 Okinawan Japanese subjects aged 25–94 years. Leukocyte telomere length was determined by Southern blot analysis of terminal restriction fragments. FOXO3 and APOE genotyping was performed by PCR.

Results: We found telomere shortening of ~42 bp/yr of telomeres in 84 subjects with the TT genotype of FOXO3 single nucleotide polymorphism rs28802292, but no telomere attrition of the longevity-associated FOXO3 G allele. Telomerase enzyme activity did not differ between FOXO3 genotypes. Genetic variation in APOE is associated with longevity and cardiovascular disease. Telomere length did not, however, differ between APOE allele ε2 carriers, subjects with the ε3/ε3 genotype and allele ε4 carriers.

Conclusion: Protection against telomere attrition may be one way by which longevity associated genotype(s) of FOXO3 increase health span and lifespan.

FOXO3: LONGEVITY INTERACTOME ON HUMAN CHROMOSOME 6

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Background: Minor alleles of several FOXO3 polymorphisms are associated with increased lifespan, as well as lower blood pressure, less hypertension and reduced risk of death from coronary artery disease.

Aims: To determine the local genomic mechanisms by which FOXO3 exerts its health benefits.

Methods: Using leukocyte DNA from long-lived American subjects of Japanese ancestry we sequenced 7.2 Mb of chromosome 6q21 DNA surrounding FOXO3. SNPs were genotyped by Genechip®. The WashU Epigenome Browser public database and the program Juicebox were used to determine contact points.

Results: We identified all SNPs in FOXO3 and showed 41 were highly significant for longevity. Thirteen of these had predicted alterations in transcription factor binding sites. Those SNPs appeared to be in physical contact, via RNA polymerase II binding and chromatin looping, with sites in the FOXO3 promoter, and likely function together as a cis-regulatory unit. The SNPs were in a high degree of linkage disequilibrium in the Asian population, in which they define a specific longevity haplotype that is relatively common in Asians. The haplotype was less frequent in whites and virtually non-existent in blacks. We identified distant contact sites in the FOXO3 promoter, that when exposed to statins, by virtue of its central location in chromatin configuration, may alter the expression of neighboring genes on other chromosomes.

Conclusion: We identified genotype-specific mechanisms affecting FOXO3 expression. Protective genotypes of FOXO3 form distinct configurations within the gene itself and between neighboring genes. FOXO3 is at the hub of an interacting set of genes on chromosome 6 involved in cell and disease protection.

ASSOCIATION OF IDIOPATHIC INFLAMMATORY MYOSITIS WITH STATIN USE: A POPULATION BASED CASE CONTROL AND ECLOGICAL STUDY
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Background: Statins are widely prescribed for cardiovascular risk reduction. The muscular adverse effects associated with statin use, including myalgia and rhabdomyolysis are well recognized. Idiopathic inflammatory myositis is a rare, severe, debilitating condition, most commonly presenting with painless proximal limb girdle weakness. It requires aggressive immunosuppressive therapy and may result in permanent disability and even death. The relationship between statin use and idiopathic inflammatory myositis is less certain.

Aims: To examine the association between histologically confirmed idiopathic inflammatory myositis and prior exposure to statins.

Methods: A retrospective population based case-control study was conducted between 1990 and 2014 of all histologically confirmed cases of inflammatory myositis in adults aged 40 years and older from the South Australian Myositis Database. Data on exposure to statins were compared to controls matched 3:1 on age (within two years) and gender from the North West Adelaide Health Study. The prevalence and odds ratio (95% CI) for statin exposure were calculated.

Results: A total of 221 cases and 662 controls were included in the study. Polymyositis (n=87, 39.4%) was the most common type identified, followed by inclusion body myositis (n=66, 29.9%), dermatomyositis (n=26, 11.8%), necrotising myositis (n=24, 10.9%) and non-specific chronic inflammatory myositis (n=18, 8.1%). A total of 30.8% of cases were exposed to statins, by comparison to 21.6% for controls. Calculated OR of exposure to statins was 1.6 (95% CI 1.15–2.27; P=0.00051) and with stratification of the analysis by time of exposure the OR increased to 2.11 (95% CI 1.33–3.53; P=0.0015). The majority of cases were not exposed to concomitant interacting medications (CYP3A4 inhibitors).

Conclusion: Patients with histologically confirmed inflammatory myositis had an increased likelihood of prior statin exposure compared with a control population. This warrants further investigation in view of the increasing population exposure to statins and the severity of the disease outcome.

RELATIONSHIP BETWEEN SLEEP AND CARDIOMETABOLIC FACTORS IN YOUNG ADULTS
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Background: Several studies have shown a relationship between sleep (especially duration) and poor cardiometabolic health in young adults. Individuals with short sleep duration have reportedly increased body mass index (BMI), waist circumference and blood pressure. However other studies have not reported such a relationship. Reasons for the discrepant findings might relate to the use of subjective methods of sleep measurement, which can be influenced by recall bias.

Aims: To examine the relationship between objective sleep characteristics and cardiometabolic risk factors in participants from the Western Australian Pregnancy Cohort (Raine) Study at 22 years of age.

Methods: Demographic, lifestyle and medical questionnaires were obtained from 975 participants that attended the Centre for Sleep Science, at the University of Western Australia. Participants were required to wear a wrist accelerometer (GTX3) continuously for 7 days and complete a sleep diary. Raw accelerometer data were recorded in one-minute periods. Data were downloaded and analysed using the Actilife software (Actigraph 2012, Actilife 6.8). Derived measurements included total sleep time, sleep efficiency, latency and Wake After Sleep Onset. Linear regression analyses were used to assess the relationships between sleep and cardiometabolic measures.

Results: 227 males and 684 females had normal weight. More males were categorized as overweight and pre-hypertensive compared to females (P<0.001). No significant association with sleep (quantity and quality) was detected in either univariate or multivariate linear regression (adjusting for gender, smoking, alcohol intake and physical activity) for any cardiometabolic variables. There was no significant association between sleep (quantity and quality) and glucose, insulin, lipids and hs-CRP.

Conclusion: No association was observed between objective sleep characteristics and cardiometabolic risk factors in the study population. More studies are, however, required in this age group to verify these findings.

CARDIOVASCULAR DISEASE, MEDICATIONS, AND HEAT: WHAT PRECAUTIONARY ADVICE IS AVAILABLE?
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Background: Global temperatures are rising, increasing the probability of population exposure to extreme heat events. Patients with cardiovascular disease may be at increased risk during extreme heat events, and cardiovascular medications may exacerbate this risk, for example, through dehydration and electrolyte imbalance, including hypotension. Normal cardiovascular adaptation to severe heat stress can involve an increase in cardiac output (CO) by up to 20 L/min and a shift of heated blood from the core to the peripheral circulation. An inability to increase CO results in impaired heat tolerance and increased susceptibility to heat stroke.

Aims: To review current evidence on how health professional resources in relation to heat-related precautionary advice for cardiovascular disease management and people prescribed cardiovascular medications.

Methods: We conducted a content analysis of the following: (1) Australian Therapeutic Guidelines Cardiovascular Version 6; (2) Australian Medicines Handbook 2015; (3) Australian Heart Foundation Guidelines; (4) Approved Product Information for specific drugs (i.e., atenolol, metoprolol, frusemide, spironolactone, glyceryl trinitrate, perindopril, irbesartan, amlodipine, atorvastatin). These resources were searched manually for the following terms: “heat,” “weather” and “season.”

Results: No advice was found for health professionals regarding the potential effects of exposure to extreme heat in patients with cardiovascular disease, nor precautionary advice for people prescribed cardiovascular medications, except for generic storage of medicines advice.

Conclusion: Precautionary advice regarding the effects of heat in patients with cardiovascular disease and use of cardiovascular medications is not generally available.

Aortic SBP variability was highly correlated with peripheral SBP variability (R²=0.96, P<0.001). Periphal SBP variability was, however, greater than aortic SBP variability at higher levels of variability (slope=0.94). The same trend was seen in BRS calculated by the sequence technique, with BRS calculated using the peripheral pulse being correlated with calculation by the aortic pulse (R²=0.92, P<0.001) but higher at higher values of BRS (slope=0.83). Spectral techniques of BRS estimation showed the same trend but the overestimation was greater in the low frequency range (0.05–0.15 Hz, slope=0.67, R²=0.93, P<0.001) than in the high frequency range (0.15–0.4 Hz, slope=0.86, R²=0.98, P<0.001).

Conclusion: Given the heart rate–dependent amplification of the arterial pulse between the aorta and peripheral sites, both SBP and BRS calculated using the peripheral pulse differ from that calculated using the aortic pulse, with the magnitude of the difference being greater at higher values of SBP and BRS.

EFFECTS OF MOXONIDINE AND LOW-CALORIE DIET IN OVERWEIGHT MALES

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Background: Elevated sympathetic nervous system activity in overweight individuals is believed to play a detrimental role in metabolic and cardiovascular health in early adulthood.

Aims: To compare the effects of a treatment with either a low-calorie diet (LCD), a sympatholytic agent or a combination of both on hemodynamic and metabolic parameters and renal and endothelial function.

Methods: Overweight (body mass index > 25 kg/m²) young (18–30 years), otherwise healthy males were randomly allocated to receive either a low calorie diet (LCD, n=10), moxonidine (MOX; n=10; 0.4 mg/day), a combination of both (LCD+MOX, n=11) or to act as controls (CONT; n=8) for a period of 6 months. Muscle sympathetic nerve activity (MSNA) was measured by microneurography, endothelial function was assessed using digital pulse tonometry, and renal function was assessed using creatinine clearance derived from the Cockcroft Gault formula before and after intervention.

Results: Weight loss occurred in the LCD and LCD+MOX (–7.5±1.9 and –7.6±1.9 kg). MSNA was significantly decreased in the LCD, MOX and LCD+MOX groups (–14±3, –14±3 and –14±2 bursts per 100 heartbeats, respectively) and this was associated with a decrease in systolic blood pressure (–8.9±3.3, –9.8±5.5 and –9.0±3.2 mmHg, respectively). All other metabolic parameters for the LCD, CON and MOX groups remained unchanged. Endothelial function remained unchanged in all groups. In the LCD+MOX group, additional benefits included decreased waist circumference (–8.3±1.9 cm), decreased total cholesterol (–0.78±0.23 mmol/l), LDL cholesterol (–0.49±0.16 mmol/l), fasting insulin (–6.5±2.8 mmol/l) and attenuated glomerular hyperfiltration (from 187±4 to 167±4 mL/min).

Conclusion: The addition of moxonidine to a LCD may have beneficial effects on the metabolic profile and renal function of overweight young males.

THE TRANS-GENERATIONAL OBESEITY RELATED HYPERTENSION: NEURONAL PLASTICITY

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Background: Obesity during pregnancy is associated with a greater risk of developing obesity and hypertension in the offspring later in life. The ventromedial hypothalamus (VMH) is a key centre of energy homeostasis, hemodynamic and sympathetic tone to renal vasculature. It is possible that exposure to over-nutrition during development alters the activity of the neurons in the VMH, amplifying sympathetic output leading to hypertension in the offspring. We assessed the contribution of leptin and melanocortin (MC) signaling pathway in the VMH of adult offspring that were born from obese mothers.

Aims: To determine whether maternal obesity plays a role in programming leptin and melanocortin signaling pathway in the VMH of adult offspring.

Methods: Female New Zealand White rabbits were fed a high fat diet (13%: mHFD) or a control diet (4%; mCD) during pregnancy and lactation. Offspring received CD after weaning. All offspring received a VMH cannula and a renal nerve recording electrode. Experiments were conducted in conscious rabbits. Systolic blood pressure (SBP), heart rate (HR) and RSNA were measured. Rabbits received increasing doses of α-melanocortin stimulating hormone (α-MSH; 0.3 and 1 nmol), a melanocortin receptor antagonist (SHU9119; 0.02 and 0.04 nmol) or leptin receptor antagonist (5 and 10 μg).

Results: mHFD rabbits exhibited higher MAP and RSNA than mCD rabbits (P<0.05). mCD did not respond to any drug injections into the VMH. Aims: To ascertain whether there is a difference between SBP variability and BRS estimated using a peripheral pulse or a derived, central aortic pulse.

Methods: Continuous finger blood pressure (Nexfin, Edwards Life Sciences) and electrocardiogram were measured in 33 subjects (age 20–66 years; 17 female) over a 10-minute interval in the supine position. A generalized transfer function was used to calculate central aortic pressure from the finger blood pressure waveform. BRS was estimated using both the sequence technique and spectral analysis and BRS variability was calculated, all using both the finger and the calculated aortic blood pressure.

Results: Aortic SBP variability was highly correlated with peripheral SBP variability (R²=0.96, P<0.001). Periphal SBP variability was, however, greater than aortic SBP variability at higher levels of variability (slope=0.94). The same trend was seen in BRS calculated by the sequence technique, with BRS calculated using the peripheral pulse being correlated with calculation by the aortic pulse (R²=0.92, P<0.001) but higher at higher values of BRS (slope=0.83). Spectral techniques of BRS estimation showed the same trend but the overestimation was greater in the low frequency range (0.05–0.15 Hz, slope=0.67, R²=0.93, P<0.001) than in the high frequency range (0.15–0.4 Hz, slope=0.86, R²=0.98, P<0.001).

Conclusion: Given the heart rate–dependent amplification of the arterial pulse between the aorta and peripheral sites, both SBP and BRS calculated using the peripheral pulse differ from that calculated using the aortic pulse, with the magnitude of the difference being greater at higher values of SBP and BRS.
Background: There is clinical interest in automated in-clinic blood pressure (AutoBP) as an alternative to traditional BP (ABP). AutoBP involves repeated, unobserved clinical BP measures, but there is variability among protocols regarding the number of readings and measurement duration. The best AutoBP protocol is unknown but needed to be determined for clinical feasibility.

Aims: To determine the optimal AutoBP protocol that has best concordance with daytime ABP and can be achieved in the shortest duration with the fewest measures.

Methods: Patients (n=117; mean age 61.5±12.5 years; 52.5% female) referred to a specialist BP clinic underwent AutoBP in a quiet room alone. Eight BP measurements were taken starting immediately after sitting and then at 2-minute intervals (15 minutes total). The optimal AutoBP protocol compared with daytime ABP was defined by the smallest mean difference and highest intra-class correlation coefficient (ICC). The same BP device (Mobil-o-graph, EM) was used for both AutoBP and daytime ABP.

Results: Average 15-minute AutoBP and daytime ABP were 138.4±18.1/84.8±12.2 and 140.9±15.2/86.2±10.6 mmHg, respectively. The optimal AutoBP protocol was derived within six minutes by the average of two measures taken after two and four minutes of seated rest (ystolic BP; mean difference = 0.3 (95%CI –3.0, 2.4) mmHg; P=0.84; ICC=0.80; diastolic BP; mean difference = 0.62 (95%CI –2.1, 1.1) mmHg; P=0.60; ICC=0.85). AutoBP measures taken before six minutes tended to overestimate daytime ABP, but measures taken after this time underestimated daytime ABP (whether as a single BP or the average of more than one BP reading).

Conclusion: A clinically feasible AutoBP protocol that best concords with daytime ABP is achievable within a short duration and using a small number of BP measures.

CAN LONG-TERM ENDURANCE EXERCISE CHANGE microRNA PROFILES AND PREVENT CARDIOVASCULAR DISEASE?

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Background: Exercise improves cardiovascular fitness and reduces the incidence of cardiovascular disease (CVD). MicroRNAs (miRs) are small non-coding RNA molecules that regulate gene expression and cardiovascular physiology.

Aims: We investigated the effects of exercise on microRNA signatures in leukocytes.

Methods: We sequenced total microRNAs from leukocytes of 12 long-term endurance athletes and 12 healthy age-matched controls using the Illumina TruSeq Small RNA library prep on MiSeq. Our findings were validated in a larger group of 68 athletes and 58 controls using qPCR. We also investigated microRNA abundance before and after a short bout of exercise leading to exhaustion in 15 healthy individuals.

Results: Mature microRNA miR-30e was up-regulated in athletes in our total microRNA investigation (P<0.05; FC=8.6); validation study (P<0.05; FC=1.55) and after a short bout of exercise (P<0.05; FC=1.43); miR-30e is down-regulated in cardiomypathies and the up-regulation of this microRNA in athletes could be cardioprotective and caused by exercise. Three novel microRNAs were only found in athletes and one in controls. The possible targets for these microRNAs are still unknown and need to be investigated.

Conclusion: Long-term endurance and short strenuous exercise influences microRNA profiles helping prevent or protect against CVD.

HMG DENSITY PROTEINS AND PRESSURE-INDUCED INFLAMMATION

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Background: In Australia, cardiovascular disease accounts for ~50,000 deaths/year. Notably, 64% of the adult population have >3 modifiable risk factors for cardiovascular disease (CVD). Accordingly, assessment of absolute CV risk, based on the patient’s gender, age, systolic blood pressure (BP), smoking status, total and high density lipoprotein (HDL)-cholesterol, type 2 diabetes and the presence of left ventricular hypertrophy is widely adopted. Although the goal is to reduce absolute CV risk, this is usually by managing individual risk factors such as high BP and lipid levels. Since both have a continuous association with CVD events, moderate alterations in several factors may however be more effective than a major change in any one factor.

Aims: To explore whether promoting cholesterol efflux or uptake influences pressure-induced leukocyte adhesion.

Methods: Carotid arteries were isolated from male 8 week-old Sprague Dawley rats and mounted on a specialized pressure myograph to record leukocyte adhesion in real time. Inflammation was induced by the exersion of high intraluminal pressure after incubation (1 h) or HDL (50 μg/ml), oxidized LDL (oxLDL; 50 μg/ml) or control. Adhesion molecule and inflammatory cytokine gene expression (intercellular adhesion molecule-1 [ICAM-1], monocyte chemotactic protein-1 [MCP-1] and interleukin-6 [IL-6]) was assessed by real time-PCR.

Results: High intraluminal pressure (120 mmHg) induced an increase in leukocyte adhesion to the endothelium, indicative of an inflammatory response (0 vs. 120 mmHg; n=12; 18±4 leukocytes per field of view; n=2; 14±7 leukocytes per field of view; n=2; 24±7 leukocytes per field of view; n=2; 39±10 leukocytes per field of view; n=2; 41±15 leukocytes per field of view; n=2). HDL had no statistically significant effect on unpressurized vessels. However, in vessels pre-incubated with HDL for 1 h prior to pressurization, there was a significant reduction in leukocyte adhesion (120 mmHg vs. HDL + 120 mmHg: 18±4 vs. 8±2 leukocytes per field of view; n=4; P<0.05). Measuring the expression of endothelial adhesion markers mirrored this effect. Interestingly, oxidized low density lipoprotein (oxLDL) augmented leukocyte adhesion and endothelial activation.

Conclusion: We found that HDL reduced high intraluminal pressure-induced inflammation in rat carotid arteries, while oxLDL potentiated inflammation. This study suggests that alterations in lipoprotein species influence the inflammatory response of vessels in the setting of hypertension.

PLASMA ACE2 ACTIVITY IS ELEVATED IN PATIENTS WITH SIGNIFICANT CORONARY ARTERY DISEASE, BUT IS NOT ASSOCIATED WITH LONG-TERM MAJOR ADVERSE CARDIOVASCULAR EVENTS


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Background: Angiotensin converting enzyme 2 (ACE2) is an important counter-regulator of the renin-angiotensin system. In patients with heart failure, elevated plasma ACE2 activity predicts adverse outcomes. It is unknown whether plasma ACE2 activity is elevated in patients with significant coronary artery disease (CAD) or if it is associated with major adverse cardiovascular events (MACE).

Aims: To determine whether plasma ACE2 activity is increased in patients with CAD and if levels predict MACE.

Methods: Plasma ACE2 activity was measured in a prospectively recruited cohort of patients (n=77) with significant CAD (determined by coronary angiography) and compared to healthy controls (n=17). Time to first occurrence of MACE (cardiovascular death, myocardial infarction, stroke, heart failure) was investigated in the CAD cohort over a mean follow-up period of 5.3±1.6 years.

Results: The median (25th, 75th percentiles) plasma ACE2 activity was significantly higher (P<0.001) in patients with CAD compared to healthy controls (9.2 [6.9, 12.5] pmol/ml/min). Among the patients with significant CAD, 50 patients (65%) presented with an acute coronary syndrome (ACS) and 27 (35%) had stable angina. Plasma ACE2 activity was not significantly different between those presenting with ACS compared to stable angina (9.9 [2.3, 15.1] vs. 9.6 [3.1, 14.6] pmol/ml/min; P=0.69). There were 31 MACE (40%) over the follow-up period. On Kaplan-Meier analysis, patients with ACS had an increased incidence of MACE compared to those with stable angina (log-rank test, P=0.008). On Cox proportional hazard analysis, troponin level >99 percentile (HR 3.7; 95% CI 1.3–10.8; P=0.017) was the only independent predictor of MACE after adjustment for age, log-transformed plasma ACE2 activity, abnormal renal function and left ventricular ejection fraction.

Conclusion: Plasma ACE2 activity is increased in patients with significant CAD, but is not associated with long-term adverse cardiovascular outcomes. Further clinical studies are needed in patients with non-significant CAD to determine if plasma ACE2 activity is increased, and to determine whether ACE2 predicts adverse cardiovascular events in patients with early atherosclerosis.

INCREASED LARGE ARTERY STIFFNESS AND IMPAIRED BAROREFLEX SENSITIVITY INDEPENDENTLY PREDICT EXAGGERATED EXERCISE BLOOD PRESSURE: A POPULATION-BASED ANALYSIS FROM THE PARIS PROSPECTIVE STUDY III

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Background: People with normal resting blood pressure (BP) but with an exaggerated BP response to submaximal exercise have adverse cardiovascular outcomes. Mechanisms of this association are unknown but could be explained through increased large artery stiffness and/or impaired baroreflex sensitivities (BRS).

Aims: To determine the association of carotid stiffness and carotid BRS with exercise BP.

Methods: Asumulatory BP was recorded at rest and immediately following an exercise step-test among 8,976 adults aged 50–75 years from the Paris Prospective Study III. Carotid artery stiffness was measured by the cross-sectional distensibility coefficient using ArtLab echotacking. BRS (low frequency gain) was calculated from carotid distension rate and heart rate using fast Fourier transformation and cross-spectral analysis. A threshold of ≥150 mmHg defined exaggerated exercise BP based on previous data. Normal resting BP was <140/90 mmHg (± hypertensive treatment).

Results: Participants with exaggerated exercise BP had significantly higher carotid stiffness (meansSD 7.35±3.18 vs 6.77±1.25 m/s; P<0.001) but lower BRS (medianIQR 0.01; 0.09/0.06.16 vs. 0.120.08.019 ms/mmHg) × 10−10, P<0.001) compared to those with normal exercise BP. Both carotid stiffness (OR 1.1495% CI 1.09, 1.20) per 1 m/s; P<0.001) and BRS (OR 0.83 (0.78.0.88) per 1 SD; P<0.001) were significantly associated with exaggerated exercise BP among participants with normal resting BP.
ARTERIAL STIFFNESS INDEX BETA AND CARDIO-ANKLE VASCULAR INDEX INHERENTLY DEPEND ON BLOOD PRESSURE, BUT CAN BE READILY CORRECTED

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Background: Arterial stiffness index (β) and cardio-ankle vascular index (CAVI) are widely accepted for quantification of the intrinsic stiffness (β0) of the blood pressure (BP)-diameter relationship. CAVI and β assume an exponential relationship between pressure (P) and diameter (d).

Aims: (1) to demonstrate that, under this assumption, β and CAVI as currently implemented are inherently BP-dependent; and (2) to provide corrected, BP-independent forms of CAVI and β.

Methods: In the intrinsic arterial BP-diameter relationship \( P = P_{d0} e^{(d/d_0)} \), usually reference \( P_{d0} \) and reference diameter \( d_0 \) are substituted with diastolic BP and diastolic diameter to accommodate measurements. Consequently, the resulting exponent (stiffness index \( \beta \)) is not equal to the intrinsic, pressure-independent \( \beta_0 \) but instead varies with BP. CAVI does not only suffer from this “reference pressure” effect, but also from a linear approximation of \( P = \beta d \). We derived corrected forms of \( \beta \) and of CAVI (CAVI0) that do not change with BP and represent the pressure-independent \( \beta_0 \). To further substantiate the BP effect on CAVI in a typical follow-up study, we realistically simulated patients (n=161) before and following BP-lowering “treatment” (assuming no follow-up change in intrinsic \( \beta_0 \) and therefore in actual \( P-d \) relationship).

Results: As an example, in a person with \( \beta_0 = 7 \), an increase of systolic/diastolic BP from 110/70 to 170/120 mmHg increased \( \beta \) by 1.8% and CAVI by 1.5%. In our simulated study patients, lowering BP from 160/117 to 170/120 mmHg instead decreased \( \beta \) (P<0.001) resulted in a significant CAVI decrease (8.1±2.0 to 7.7±2.1; P=0.008); CAVI, did not change (9.8±2.4 and 9.9±2.6; P=0.499).

Conclusion: Stiffness index \( \beta \) and CAVI as currently implemented are inherently BP-dependent, potentially leading to erroneous conclusions in arterial stiffness trials. BP-independent forms of \( \beta \) and CAVI are presented to readily overcome this problem.

EFFECTS OF SALT SUPPLEMENTATION ON microRNA EXPRESSION IN TYPE 2 DIABETES

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Background: The benefits of salt restriction in type 2 diabetes mellitus (T2DM) have been challenged given recent observational and experimental research suggesting an association between increased cardiovascular mortality and reduced salt intake. Mechanistic studies to explain this paradoxical finding are limited. MicroRNA (miRNA) are non-coding RNAs linked to T2DM and its vascular complications. \( \text{miRNA-27a}, \text{miRNA-221}, \text{miRNA-320a} \) are considered surrogate markers for endothelial dysfunction. The effect of salt supplementation on miRNA expression has not previously been investigated in patients with T2DM. We hypothesized that salt supplementation compared to placebo would upregulate \( \text{miR-126} \) and downregulate \( \text{miRNA-221} \) and \( \text{miRNA-320a} \). Aims: To determine the effect of salt supplementation on the expression of miRNAs in patients with T2DM and habitual low-salt intake. Methods: Participants in this pilot prospective crossover study, conducted at a university teaching hospital, were recruited from a diabetes outpatient clinic. Participants (n=19) with low-normal habitual dietary salt intake determined by 24-h urinary collection (corrected sodium excretion less than 150 mmol in 2 out of 3 collections) were randomized to salt supplementation (100 mmol NaCl/24 h over 3 weeks) or placebo. Platelet-free plasma samples collected post-NaCl supplementation (n=19) and post-placebo (n=19) were analyzed for miRNA expression utilizing a protocol that was optimized for the extraction of miRNA then quantified by real-time PCR. Expression was compared to an endogenous control. Results: From the 38 patient samples that were analyzed, \( \text{miRNA-27a}, \text{miRNA-221} \) and \( \text{miRNA-320a} \) were detected at a detectable level in 18 samples from 9 patients following salt and placebo supplementation, respectively. Logarithmic transformation was applied for non-parametric data and Wilcoxon signed-rank statistical analysis was performed. No significant differences in miRNA expression between salt and placebo were demonstrated; \( \text{miRNA-320a} (P=0.59); \text{miRNA-221} (P=0.76); \text{miRNA-126} (P=0.44); \text{miRNA-221} (P=0.68) \). Conclusion: In this pilot study, we demonstrated the feasibility of detecting microRNA expression from plasma samples of patients with diabetes. However, we found no significant difference in miRNA expression with or without salt supplementation. This proof-of-concept study may provide a platform for larger studies aimed at elucidating the relationship between miRNA and vascular disease in T2DM.

HEART RATE DEPENDENCY OF ARTERIAL STIFFNESS: UNDERLYING MECHANISMS RELATED TO FREQUENCY DEPENDENCY OF ARTERIAL ELASTICITY

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Background: It has been established that the stiffness of large arteries has a dependency on acute heart rate (HR) changes. However, possible mechanisms behind this HR dependency are hard to establish.

Aims: To explore a plausible mechanism by which HR exerts an influence on arterial stiffness.

Methods: Using a computerized transmission line model of the human arterial tree, effects of HR on aortic arch to femoral pulse wave velocity (afPWV) were determined with elasticity of the arterial segments modelled with varying degrees of frequency dependence between 0 to 20 Hz. Magnitude of the elasticity was varied as a proportion of the particular segment’s static elastic modulus.

Results: In scenarios in which the arterial wall elasticity had low to zero frequency dependence, afPWV was shown to decrease with HR. As the degree of frequency dependence increased, an increase in afPWV with increasing HR was observed. The critical frequency, defined as the frequency where arterial wall elasticity reached 80% of the static elastic modulus, above which HR was shown to positively influence afPWV, was approximately 3 Hz. The change in afPWV with increasing HR plateaued at around 0.06 m/s per 10 bpm increase in HR as the degree of frequency dependence was increased to above 9 Hz.

Conclusion: The magnitude of the frequency dependency of arterial wall elasticity alters measures of large arterial stiffness across physiological ranges of HR. This could be a partial mechanism through which large artery stiffness changes with HR. Physiological studies are required to confirm this mechanism.

POWER SPECTRUM ANALYSIS AS A MEASURE OF VASCULAR REACTIVITY: EXPLORING NEURAL CONTROL OF CARDIOVASCULAR FUNCTION

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Background: Non-invasive methods are valuable in order to estimate vascular reactivity as well as study physiology and pathophysiological conditions such as heart failure. Power spectral analysis is used to convert data from the time domain into the frequency domain using a fast Fourier transform (FFT) algorithm. Vascular reactivity governs the state of dilation or constriction of muscular resistance arteries, which in turn determine blood flow to different vascular beds in response to changes detected by the autonomic nervous system. Previous work suggests that changes in total peripheral resistance and cardiac output are reflected by fluctuations in power of the low frequency band of systolic blood pressure (SBP), since this band is most affected by sympathetic blockade.

Aims: To estimate the neural control of cardiovascular function in older male heart failure patients using a power spectrum analysis between salt and placebo were demonstrated. Methods: Continuous, non-invasive blood pressure recordings were obtained using the Nexfin® apparatus in the supine and standing positions for older males (> 65 years of age) with heart failure (n=7), and in age-matched controls (n=8). Spike2 software was used to extract a waveform representing SBP variability. Power spectral analysis was used to decompose this complex, periodic waveform into its component waves using an FFT algorithm. The area under curve value for the low frequency (LF) band (0.04–0.15 Hz) was normalized against the difference between total power of the spectrum and the very low frequency band (< 0.04 Hz).

Results: Patients with heart failure displayed significantly lower LF-SBP (n=7; 22.2±1.8; P<0.05) compared with age-matched controls (n=8; 33.6±2.2). Heart failure patients also had lower mean SBP (n=7; 117.2±5.3) compared to age-matched controls (n=8; 129.6±5.3).

Conclusion: Our results indicate that the low frequency band (0.04–0.15 Hz) power of male heart failure patients is significantly lower than the control group. This suggests a decrease in efferent control of resistance arterioles. Therefore, power spectral analysis of SBP variability appears to be a powerful non-invasive tool that can be used to test vascular reactivity. We speculate that the use of this measure would be a valuable addition to available clinical measures of the success, or failure, of management of cardiovascular disease.
Sex Differences in Outcome After Permanent Focal Cerebral Ischemia

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2Background: Stroke incidence is lower in young females than age-matched males. Females appear, however, to experience worse long-term outcomes. Dampening of the immune system after stroke leaves the patient more susceptible to infections, but the effect of sex on this phenomenon is unknown. A key estrogen receptor, the G-protein coupled estrogen receptor (GPER), exhibits sex-dependent neuroprotective effects after stroke and could potentially influence immunosuppression.

Aims: To test whether a sex difference exists in outcomes after permanent focal cerebral ischemia, and if estrogen-mediated activation of GPER is involved.

Methods: Brain infarct volume and bacterial infection in lungs were assessed 24 h after permanent middle cerebral artery occlusion (pMCAO) in female (n=41) and male (n=20) mice. GPER agonist (G-1) or vehicle were administered to ovariectomized female mice immediately before induction of pMCAO and infection levels were assessed 23 h later.

Results: Higher mortality was observed in females than males (27% vs. 15%). No sex difference was detected in infarct size, mortality or bacterial infection. Stroke, however, decreased spleen weight selectively in females (F=14–28; P<0.01), G-1 worsened bacterial infection in females (n=4–7; P<0.0001) with no effect on infarct size.

Conclusion: Independent of the degree of brain injury, females suffer greater mortality after permanent focal ischemia. Furthermore, a GPER agonist increases infection levels after stroke in females in the absence of endogenous estrogen. More studies are needed to elucidate whether GPER modulation could be exploited for post-stroke neuroprotective therapy in older females.

Night-Time Ambulatory Blood Pressure is the Best Preliminary Blood Pressure Predictor of 11-Year Mortality in Older Hypertensives

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Background: Population-based and prospective observational studies have demonstrated a stronger relationship between ambulatory blood pressure (ABP) and subsequent cardiovascular events than for clinic blood pressure (BP), and some of these studies suggest that this relationship is strongest for night-time BP. Only one previous study has examined these relationships in the context of a clinical trial, demonstrating that ABP was a better predictor of outcome than clinic BP in placebo-treated but not actively-treated participants.

Aims: To test the hypothesis, in elderly hypertensives from the Second Australian National Blood Pressure Study (ANBP2), that at study entry ABP was a better predictor of long-term mortality than clinic BP.

Methods: ANBP2 was a comparative outcome trial of different BP-lowering treatment regimens in 6,083 off-treatment or previously untreated hypertensives aged 65–84 years. In the ABP sub-study, at study entry participants had ABP recordings in addition to nurse-performed standardized clinic BP measurements. Cox proportional hazards analysis with relevant adjustments assessed the relationships between both clinic BP and ABP at study entry and 11 year all-cause and cardiovascular mortality (including both in-trial and post-trial periods). Both treatment arms were pooled.

Results: Of participants, 702 out of 735 in the ABP sub-study had “successful” ABP recordings at study entry. Over a median period of 10.8 years, including 6.7 years post-trial follow-up, 167 of these 702 participants died, with 82 cardiovascular deaths. “Night” ambulatory systolic BP and pulse pressure were the best significant BP predictors at study entry of the ABP sub-study, at study entry participants had ABP recordings in addition to nurse-performed standardized clinic BP measurements.

Conclusion: In untreated or off-treatment elderly hypertensives participating in a clinical outcome trial, post-trial all cause or cardiovascular deaths were significantly related to ABP measured at study entry, particularly to night-time systolic BP and pulse pressure, but not to clinic BP.

The Involvement of Kidney DNA Methylation in Blood Pressure Regulation

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Background: Increasing evidence suggests that epigenetic modifications such as DNA methylation (5mC) is important to the development of essential hypertension, and that changes in DNA methylation of blood cells are associated with blood pressure (BP). So far, there have been no studies of epigenetic changes in the kidney – an important effector organ in BP regulation.

Aim: To compare loci-specific methylation in the kidney between normal and hypertensive subjects.

Methods: We used 96 human renal samples from the TRANScripTome of Renal, HumAn TissueE (TRANSLate) Study to measure DNA methylation. TRANSlate consists of carefully characterized collections of “apparently healthy” specimens of human kidneys. DNA was extracted from kidney tissue using the QiNasy blood and tissue Qiagen kit and lco-specific methylation status was determined using Infinium HumanMethylation 450K array (Illumina®, Australia).

Results: We found 505 (P<0.001) loci were differentially methylated between hypertensive and normotensive individuals. Included in these loci were genes previously associated to blood pressure modulation (B4T4, CASZ1, DMG1, FGXY, HEXM2, INSR, KCNJ11, KEGG, PPL, SKT1, TBC1D1, THBS2, MAP3K11, EBPHI1L1 and XKR6) in genome-wide association studies. Of these, one gene (M32C2) is associated with monogenic forms of hypertension. The variants identified implicate biological pathways related to aldosterone-regulated sodium reabsorption, vasopressin-regulated water reabsorption and vascular smooth muscle contraction.

Conclusion: DNA methylation in the kidney is an important molecular mechanism contributing to BP variation and hypertension in humans.

Quantitative mRNA Analysis of the Thiazide-Sensitive Sodium Chloride Cotransporters in Urinary Exosomes from Normal Controls and Patients with Primary Aldosteronism

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Background: The thiazide-sensitive sodium chloride cotransporter (NCC) is responsible for the major sodium chloride reabsorption in the distal convoluted tubule of the renal nephron. As an aldosterone-modulated cotransporter, NCC regulation by aldosterone remains unclear. Our preliminary data show, in humans, mineralocorticoid administration acutely increased NCC abundance, NCC phosphorylation and WNK4 abundance by 3.68-fold, 2.73-fold, and 3.23-fold, respectively (P<0.01). These results suggested that mineralocorticoid administration is associated with a rapid increase in abundance of NCC and its activated form, possibly via the WNK pathway.

Aims: To establish and evaluate a novel approach for quantification of NCC mRNA in human urinary exosomes, and to investigate the relationship between NCC mRNA expression and the increased NCC protein abundance previously reported by us to be induced by fludrocortisone administration in primary aldosteronism (PA) patients.

Methods: Morning spot urine samples from non-hypertensive volunteers were collected and stored at –80°C for method optimization. Urinary exosomes were harvested by progressive ultracentrifugation, and NCC abundance was determined and quantified by quantitative reverse transcriptase polymerase chain reaction (qRT-PCR).

Results: Among non-hypertensive volunteers, NCC mRNA analysis for NCC expression was performed. The thiazide-sensitive sodium chloride cotransporter (NCC) regulation by aldosterone remains unclear. Our preliminary data show, in humans, mineralocorticoid administration acutely increased NCC abundance, NCC phosphorylation and WNK4 abundance by 3.68-fold, 2.73-fold, and 3.23-fold, respectively (P<0.01). These results suggested that mineralocorticoid administration is associated with a rapid increase in abundance of NCC and its activated form, possibly via the WNK pathway.

Conclusion: Total RNA in human urinary exosomes has been successfully isolated by the optimized methods. This will help in the exploration of molecular mechanisms that govern NCC expression and abundance, including those induced by mineralocorticoids, and may ultimately aid in the clinical diagnosis and treatment of PA.

Changes in Left Ventricular Ejection Time But Not Peripheral Resistance Alter Carotid and Femoral Waveform Shapes, Impacting on Measured Carotid-Femoral Pulse Wave Velocity: A Modelling Study

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Background: Changes in DNA methylation (5mC) is important to the development of essential hypertension, and that changes in DNA methylation of blood cells are associated with blood pressure (BP). So far, there have been no studies of epigenetic changes in the kidney – an important effector organ in BP regulation.

Aim: To compare loci-specific methylation in the kidney between normal and hypertensive subjects.

Methods: We used 96 human renal samples from the TRANScripTome of Renal, HumAn TissueE (TRANSlate) Study to measure DNA methylation. TRANSlate consists of carefully characterized collections of “apparently healthy” specimens of human kidneys. DNA was extracted from kidney tissue using the QiNasy blood and tissue Qiagen kit and lco-specific methylation status was determined using Infinium HumanMethylation 450K array (Illumina®, Australia).

Results: We found 505 (P<0.001) loci were differentially methylated between hypertensive and normotensive individuals. Included in these loci were genes previously associated to blood pressure modulation (B4T4, CASZ1, DMG1, FGXY, HEXM2, INSR, KCNJ11, KEGG, PPL, SKT1, TBC1D1, THBS2, MAP3K11, EBPHI1L1 and XKR6) in genome-wide association studies. Of these, one gene (M32C2) is associated with monogenic forms of hypertension. The variants identified implicate biological pathways related to aldosterone-regulated sodium reabsorption, vasopressin-regulated water reabsorption and vascular smooth muscle contraction.

Conclusion: DNA methylation in the kidney is an important molecular mechanism contributing to BP variation and hypertension in humans.
Background: Large artery stiffness, a cardiovascular risk factor, is blood pressure and heart rate (HR) dependent. However, the effects of waveform input (for example, left ventricular ejection time, LVET) and wave reflection (for example, changes in peripheral resistance) are harder to elucidate.

Aims: To quantify the effect of LVET and peripheral resistance changes on carotid-femoral pulse wave velocity (cfPWV), a measure of large artery stiffness, through a computational model.

Methods: Using a transmission line model of the human arterial tree, changes in HR (60–100 bpm), LVET (0.1–0.45 s) at a fixed HR of 70 bpm, and peripheral resistance (50–150%) were modelled and the blood pressure waveform at the carotid and femoral sites generated for a static elastic modulus (non-pressure and non-frequency dependent). cfPWV was calculated using waveform shape methods as applied in the clinic (intersecting tangents, maximum systolic upstroke) and by calculation of the phase velocity.

Results: Without a dynamic elastic component of elasticity, cfPWV was not dependent on HR, but increased significantly with increasing LVET (0.17±0.22 m/s per 50 ms) when calculated using the intersecting tangents or systolic upstroke method. Correlation of cfPWV with peripheral resistance was strong, but the magnitude of the dependency was low (0.03±0.01 to 0.05±0.05 m/s per 10% increase in resistance) when calculated by the intersecting tangent or systolic upstroke method. When cfPWV was calculated using phase velocity (independent of blood pressure waveform shape), the expected zero dependency on LVET or peripheral resistance was identified.

Conclusion: Increased LVET causes changes in blood pressure waveform shape that increased cfPWV if measured by the intersecting tangents or maximum systolic upstroke methods. Changes in HR (without accounting for viscoelasticity) and changes in peripheral resistance (independent of blood pressure) either have no, or minimal, effect on measured cfPWV.