KO hearts develop significantly larger infarctions following lethal ischaemia and reperfusion (25.1±1.97% in PINK1+/+ hearts vs 38.9±3.42% ($p<0.01$) and 51.5±4.3% ($p<0.001$) in PINK1+/- and PINK1−/− hearts, respectively). Interestingly, electron microscopic images showed significantly more vacuole-like structures that contained cellular material (indicative of autophagy) in PINK1−/− hearts. We further observed that PINK1−/− hearts had significantly more Beclin1 and total LC3b than hearts from PINK1+/+ littermate controls (Beclin1: 0.674±0.065 in PINK1+/+ vs 0.85±0.019 in PINK1−/− hearts, $p<0.05$) total LC3b: 0.946±0.139 in PINK1+/+ vs 1.445±0.141 in PINK1−/− hearts, $p<0.05$; values are in arbitrary units of densitometry). In conclusion, our results suggest that during ischaemic-reperfusion PINK1 acts as an endogenous protective kinase with the regulation of mitophagy being a possible mechanism of its protection.

BAS/BSCR16 TIME-DEPENDENT CHANGES IN ATRIAL NITRIC OXIDE-REDOX BALANCE IN ATRIAL FIBRILLATION: TRANSLATIONAL RESEARCH (FROM GOATS TO HUMANS)

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Rationale Oxidative stress has an important role in atrial fibrillation (AF)-induced myocardial remodelling, suggesting that specific oxidases may represent a novel therapeutic target in AF. Here we evaluated how the duration of AF affects the level, sources and localisation of superoxide.

Results and methods Our previous work in patients with predominantly paroxysmal AF showed that increased superoxide was produced by NOX2/NADPH oxidase. Here, in patients with permanent AF (n=26) versus matched controls in sinus rhythm (n=53), increased superoxide (assessed by lucigenin-enhanced chemiluminescence and 2-hydroxyethidum detection) was maintained by mitochondrial oxidases and 'uncoupled' nitric oxide synthase (NOS), but not NOX2/NADPH oxidase; although NOX4/NADPH oxidase was upregulated (real-time RT-PCR). Immunoblotting revealed increased protein expression of the mitochondrial complexes I–V and mitochondrial antioxidant peroxiredoxin-3; NOS 'uncoupling' was associated with reduced tetrahydropterin by 40% (BH4, HPLC).In the goat, after 14 days of AF, NADPH oxidase activity and protein expression were increased in the left atrium (LA). After 6 months of AF, superoxide release was doubled in both atria and originated from mitochondrial oxidases and 'uncoupled' NOS, which was associated with ipsilaterally reduced BH4 and increased arginase activity. Manganese superoxide dismutase was reduced by 50% at this stage.

Conclusion Activation of LA NOX2/NADPH oxidase occurs early in AF and is transient, since mitochondrial oxidases and 'uncoupled' NOS account for the increased superoxide production in permanent AF in both models. This suggests that NADPH oxidases may be a valuable target for 'upstream' treatment in short-term AF, but not once AF is established.

BAS/BSCR17 MYOCARDIAL XANTHINE OXIDASE REGULATES BASAL INOTROPY IN MURINE LEFT VENTRICULAR MYOCYTES

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Xanthine oxidase (XO) is a major source of reactive oxygen species in the cardiovascular system. Enhanced XO activity in the failing myocardium has been associated with a reduction in inotropy; however, whether this association is causal remains to be established. To test this hypothesis, the effect of XO inhibition (oxypurinol, 100 μmol/l and allopurinol, 100 μmol/l) or activation (xanthine, 100 or 500 μmol/l) on cell shortening (3 Hz, 35°) was evaluated in left ventricular (LV) myocytes isolated from C56BL/6–129j mice. Similarly, LV superoxide production in the absence and presence of inhibitors of XO, NADPH oxidases (apocynin, 100 μmol/l) or nitric oxide synthases (LNAME, 1 μmol/l) was measured by lucigenin (5 μmol/l)-enhanced chemiluminescence. Oxypurinol and allopurinol significantly suppressed basal superoxide production and cell shortening (by about 20%), whereas xanthine caused a dose-dependent increase in cell shortening and superoxide production. In contrast, apocynin had no effect on superoxide release or cell shortening. Taken together, our findings indicate that superoxide production by XO exerts a tonic positive inotropic effect on murine LV myocytes, suggesting that the increase in XO activity in heart failure may be, at least in part, adaptive.

BAS/BSCR18 PROTECTION FROM DEVELOPMENT OF OBESITY IN HIGH-FAT DIET FED RATS IS ASSOCIATED WITH PRESERVATION OF THE ANTICONTRACTILE FUNCTION OF PERIVASCULAR ADIPOSE TISSUE

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Introduction In health, perivascular adipose tissue (PVAT) has an anticontractile function on adjacent small arteries. We have recently shown that adipocyte hypoxia and inflammation in obesity attenuates PVAT anticontractile function. In animals, PVAT function has only been examined in genetic models of obesity, which are rare in clinical practice.

Methods 11 Sprague–Dawley rats were fed a high-fat diet (HF; n=11) over 15–18 weeks. Seven control animals received a normal diet. Weight and blood pressure were monitored. The HF rats were split into two groups: (a) diet-induced obese (DIO; n=6): significantly gained weight after a 10-week period, and diet resistant (DR; n=5): weight comparable to control group. Mesenteric arteries were studied using wire myography with construction of cumulative dose responses to noradrenaline, with and without PVAT intact.

Results The weight and systolic blood pressure for DIO were significantly increased compared with the controls (systolic BP: control: 124%±4; DR: 138%±8; DIO: 150%±3 $p<0.05$). The contractile responses of vessels with intact PVAT were significantly different from vessels without PVAT in control ($p<0.001$—multiple ANOVA) and DR ($p=0.001$—multiple ANOVA) groups. In DIO, the dose–response curves for vessels with intact PVAT and without PVAT were not significantly different ($p=0.210$—multiple ANOVA).

Conclusion The anticontractile function of PVAT was preserved in DR, but partially lost in DIO. This suggests that weight gain rather than diet itself initiates PVAT damage, which is associated with hypertension. This is the first animal model of environmental obesity in which PVAT function has been studied.

BAS/BSCR19 VISUALISING INFLAMED ATHEROSCLEROTIC PLAQUES: MOLECULAR IMAGING USING MRI AND TARGETED ULTRASMALL SUPERPARAMAGNETIC PARTICLES OF IRON OXIDE

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Introduction There are currently no clinical imaging techniques available to assess the degree of inflammation associated with

atherosclerotic plaques. This study aims at visualising and characterising atherosclerosis using targeted ultrasmall superparamagnetic particles of iron oxide (USPIO) as an MRI probe for detecting inflamed endothelial cells and inflamed atherosclerotic plaques.

Method The in vitro study consists of detection and characterisation of inflammatory markers on activated endothelial cells by immunocytochemistry and anti-E-selectin antibody-conjugated USPIO. The ex vivo stage involves characterisation of inflammatory markers on human atherosclerotic plaques.

Results We have established an in vitro cellular model of endothelial inflammation induced with tumour necrosis factor α. We have confirmed the inflammation of endothelial cells with both immunocytochemistry and MRI. We can also image the inflammation of human atherosclerotic plaques by ex vivo MRI.

Conclusion We successfully developed an in vitro model to detect and characterise inflamed endothelial cells by immunocytochemistry and MRI. We can also image the inflammation of human atherosclerotic plaques by ex vivo MRI. This will allow us to develop agents and protocols for imaging vascular inflammation in atherosclerosis in the future. This pilot study will form the basis for a translational study to provide clinicians with a novel tool for in vivo assessment of atherosclerosis.

BAS/BSCR20 THE ROLE OF A GAB1—TRIBBLES 2 INTERACTION IN PHOSPHOINOSITIDE-3-KINASE, AKT/PKB CASCADE REGULATION AND CELLULAR MORPHOLOGY

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Apoptosis is a key event in atherosclerotic plaque formation. The phosphoinositide-3-kinase (PI3K) cascade is involved in many cellular activities in plaques, such as migration and cell survival. Our previous data have identified an interaction between Tribbles 2 (Trb2) and signalling adaptor molecule Grb2 associated binder protein (Gab1). The functional consequences of this interaction are unknown. Gab1 interacts with the p85 domain of PI3K to mediate downstream activation of the Akt/PKB anti-apoptotic signalling pathway. We examined whether tribbles, a new family of signalling regulators, link with PI3K to control activation of Akt and potentially inhibit apoptosis.

HEK293 cells were transfected with Trb2 and mutant or wt Gab1 and formation of Trb2/Gab1 complexes was quantified using a yellow fluorescent protein-based protein fragment complementation assay. We show that overexpression of the PI3Kδ and β (catalytic) and the PI3Kα (regulatory) subunits leads to an increase in Trb2—Gab1 interaction (relative binding intensity 2.48%± SEM vs 3.9%± SEM, $p<0.05$, and 2.52%± SEM vs 5.3%± SEM, respectively). These data suggest that Trb2/Gab1 binding is modulated via a PI3K dependent feedback loop and raise the possibility that that Trb2 may act as a co-regulator of Gab1. In addition, we show that Gab1 has an effect on cell morphology upon PI3K cascade activation, and this morphological consequence is potentiated further in the presence of Trb2. These findings in turn suggest that the Gab1—Trb2 interaction may participate in controlling cell survival and morphology and potentially, atherosclerotic plaque development or rupture.

BAS/BSCR21 AKT PROTECTS THE HEART BY PROMOTING MITOCHONDRIAL FUSION

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Background Mitochondria change their morphology by undergoing 'fusion' and 'fission' to generate elongated and fragmented mitochondria, respectively. We hypothesised that inducing mitochondrial fusion protects the heart against ischaemia-reperfusion injury (IRI), and that this mechanism underlies the cardioprotection elicited by the pro-survival kinase, Akt.

Methods/results Inducing mitochondrial fusion in HL-1 cells (a cardiac cell line), using mitochondrial fusion proteins and a pharmacological inhibitor of a mitochondrial fission protein (called mdivi-1) delayed the opening of the mitochondrial permeability transition pore (mPTP, a critical mediator of IRI) and reduced cell death following IRI. Overexpressing Akt induced mitochondrial elongation (49.0±5.8% control vs 72.8±5.0% Akt; N=4 experiments:*$p<0.05$), delayed mPTP opening (twofold; N=4 experiments: $p<0.05$); and reduced cell death following IRI (64.9±5.6% control vs 34.2±1.2% Akt; N=4 experiments:*$p<0.05$). Treatment with the cytokine, erythropoietin (EPO, 10 U/ml), also induced mitochondrial elongation (30.0±3.5% with control vs 67.0±3.4% for EPO; N=4 experiments:*$p<0.05$), delayed mPTP opening (twofold; N=4 experiments: $p<0.05$); and reduced cell death following IRI (43.1±2.7% control vs 17.0±2.7% EPO; N=4 experiments:*$p<0.05$). Finally, elongated mitochondria extending 4—6 μm in length were observed in adult rodent hearts using electron and confocal microscopy. Treatment with mdivi-1 increased the number of elongated mitochondria and protected against IRI, as shown by reduced cell death in adult cardiomyocytes following IRI, and reduced myocardial infarct size in the in vivo murine heart.

Conclusions Inducing mitochondrial fusion protects the heart against IRI by delaying mPTP opening. Akt activation also promotes mitochondrial fusion, delays mPTP opening and protects against IRI. These results suggest that modulating mitochondrial morphology may be a novel strategy for cardioprotection.

BAS/BSCR22 TIME SERIES ANALYSIS OF ACUTE CORONARY SYNDROME FROM PERIPHERAL WHOLE BLOOD USING AFFYMETRIX GENECHIP ARRAYS

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Introduction Acute coronary syndrome (ACS) is the cause of over 114 000 UK hospital admissions[1] and a cost to the NHS of over £3.9 billion every year.[2] Advances in microarray technology allow a detailed understanding of genome-wide expression profiles of pathological processes. We hypothesised that analysis of ACS, at the time of an acute event and throughout recovery, would provide insight into pathology, as well as identify genes as potential drug targets and both diagnostic and prognostic markers.

Methods 50 patients presenting with chest pain consistent with ACS were recruited within 48 h of admission. 3 ml of peripheral whole blood was collected using Tempus RNA tubes at days 1, 3, 7, 30 and 90. Total RNA was extracted, cleared of globin mRNA and arrayed using Affymetrix HG_U133 plusv.2 GeneChips. Data were analysed using open source software PUMA.

Results We used principal component analysis (PCA) to visualise the data. With clinical information incorporated, it was found that the data discriminated between patients, putting them into troponin-positive and troponin-negative groups across all time points. Hierarchical clustering, comparing the expression profiles between groups, identified different clusters of genes that increased in expression over time in the troponin-positive group. Pathway analysis of the clusters showed overexpression of Rho GTPase cytoskeletal, endothelin signalling, integrin signalling, G-protein signalling and inflammation-mediated pathways.