SMC were isolated via enzymatic dissociation of endarterectomy specimens and using immunomagnetic beads (Miltenyi). Commercially available aortic medial SMC from six healthy donors were purchased from Promocell. Cells were used consistently at passage 3. We used two-dimensional electrophoresis with digital image analysis (SameSpots, Non-linear Dynamics) and tandem mass spectrometry to detect changes in proteome of atherosclerotic SMC.

Results Analysis of 2D gel images revealed 29 proteins with a statistically significant difference in expression between medial and plaque SMC ($p<0.05$). Plaque SMC had decreased expression of mitochondrial protein ATP synthase subunit β but an increase in the oxidised form of peroxiredoxin-4, suggesting decreased mitochondrial function, possibly owing to oxidative stress. Glycolytic enzyme pyruvate kinase was also increased by 50% in plaque SMC. Furthermore, differences in protein expression between SMC from symptomatic and asymptomatic patients were also found. Plaque SMC from symptomatic patients exhibited increased expression of heat shock protein-60 and decreased levels of the anti-inflammatory protein annexin I ($p<0.05$), compatible with a proinflammatory behaviour. These findings were confirmed by immunoblotting.

Conclusions Our data demonstrate that plaque-derived SMC are exposed to higher levels of oxidative stress than control SMC. Differences between SMC from symptomatic and asymptomatic patients appear to reflect proinflammatory changes associated with plaque instability.

BAS/BSCR13 INCREASED PLASMINOGEN ACTIVATOR INHIBITOR-1 MAY EXPLAIN DEXAMETHASONE-INDUCED THROMBOSIS AS SITE OF INTRALUMINAL WIRE INJURY

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We have previously shown that high-dose glucocorticoid treatment reduces neointimal proliferation following arterial injury in mice, but is associated with increased local thrombosis. Clinically, glucocorticoid excess, either in Cushing's syndrome or with chronic treatment, is associated with increased coagulation and decreased fibrinolysis. The few animal (rat) studies which have investigated these effects support the conclusion that glucocorticoid treatment reduces fibrinolysis. This study aimed to determine the influence of glucocorticoid administration on the thrombotic potential in mice.

Male C57Bl/6J mice (aged 10–12 weeks) received either vehicle or dexamethasone (dex; 0.1 or 0.8 mg/kg/day) orally for 5 weeks (n=8/group). Tail tip bleeding time was reduced by high-dose dex (52.9±5.6 s) compared with vehicle (87.1±13.6 s; $p<0.05$). High-dose dex increased plasminogen activator inhibitor-1 (PAI-1; 139.5±15.1% vs vehicle 100.0±10.7%; $p<0.05$ and decreased tissue plasminogen activator (tPA; 55.9±4.9% vs vehicle 100.0±10.3%; $p<0.05$) mRNA levels in the heart. In addition, high-dose dex increased total PAI-1 (3.53±0.59 ng/ml vs vehicle 0.96±0.17 ng/ml; $p<0.001$) and active PAI-1 (0.92±0.09 ng/ml vs vehicle 0.31±0.07 ng/ml; $p<0.001$) plasma antigen levels. High-dose dex did not alter platelet activation as measured by p-selectin expression using flow cytometry. Low-dose dex had no effect on any parameters described.

Dexamethasone-induced thrombosis at the site of intraluminal wire injury may be attributable to alterations in the endogenous fibrinolytic system rather than changes in platelet activity. These results suggest that beneficial inhibition of neointimal proliferation mediated by systemic glucocorticoid administration is negated in part by changes in fibrinolysis leading to large, semi-occlusive thrombi formation at the site of injury.

BAS/BSCR14 LIPIDOMIC PROFILING OF HUMAN ATHEROSCLEROTIC PLAQUES

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Rationale Lipid accumulation in arteries is the hallmark of atherosclerosis. Yet, a comparative lipid analysis of human plaques using state-of-the-art mass spectrometry techniques has not been performed.

Methods and results Comparative lipid profiling of radial arteries and carotid endarterectomies was performed by ultra performance liquid chromatography mass spectrometry. Lipid species were subsequently identified by shotgun lipidomics using a triple quadrupole mass spectrometer. Twenty-two different scan modes in positive and negative ion mode plus additional scans for different fatty acids resulted in the detection of 149 lipid species from nine different classes. Twenty-nine lipid species were only found in endarterectomy specimens. The main qualitative differences between control and diseased arteries were identified among lysophosphatidylcholines, sphingomyelins and cholesteryl esters (CE), including two oxidised CE species. Quantitative mass spectrometric analysis showed that the average concentration of CEs exceeded 30 pmol/μl in atherosclerotic plaques compared with only 0.3 pmol/μl in control arteries. Further analyses focused on plaques from symptomatic and asymptomatic patients and stable and unstable plaque areas from the same lesion. Principal component analysis confirmed that among all the lipids analysed most of the information was in the CEs but no clear discrimination could be obtained between patients (n=24).

Conclusions This study provides the most comprehensive lipid analysis of human atherosclerosis to date. Comparative lipid profiling of control and diseased arteries resulted in the identification of plaque-specific lipids, but failed to unambiguously identify vulnerable lesions.

BAS/BSCR15 THE ROLE OF PINK1, A MITOCHONDRIAL PRO-SURVIVAL KINASE, IN MYOCARDIAL ISCHAEMIA-REPERFUSION

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PINK1, a kinase localised mainly in mitochondria, can protect neurons against oxidative damage and apoptosis by maintaining mitochondrial structure and function. Although PINK1 is highly expressed in the myocardium, there are no studies investigating the role of this kinase in the heart. We hypothesised that PINK1 could protect cardiomyocytes from ischaemia-reperfusion (I-R) injury and therefore, PINK1 downregulation would be detrimental to the ischaemic-reperfused heart. Hearts isolated from PINK1+/+, PINK1 +/- and PINK1−/− mice were perfused in a Langendorff constant pressure system and subjected to 35 min global normothermic ischaemia and 30 min reperfusion. Infarct size was measured, using triphenyltetrazolium chloride (TTC) staining, and expressed as percentage of the myocardium at risk (I/R%). Electron microscopy was used to investigate ultrastructural changes in cardiomyocytes, and the expression of autophagic markers such as Beclin1 and LC3b was measured in PINK1−/− hearts. Our data show that the PINK1

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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

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