of the endothelium reduces bioavailability of the anti-atherosclerotic signalling radical nitric oxide (NO). We explored the effects of increasing insulin signalling in the endothelium, using novel transgenic mice, over-expressing Type A human Insulin Receptor (HIRECO) in the endothelium, driven by the Tie-2 promoter-enhancer.

Methods Semi-quantitative RT-PCT was carried out on various tissues and isolated endothelial cells from lungs to confirm significant levels of human insulin receptor mRNA while the protein expression was confirmed by western blotting on aortic sections or endothelial cells. Lucigenin-enhanced chemiluminescence was exploited to measure superoxide anion levels while; vasomotor functions were assessed in thoracic aortic rings mounted in organ baths.

Results No significant changes in morphological features, metabolic phenotypes or blood pressure regulation were observed between the HIRECO and wild type (WT) littermates. However, plasma insulin levels were similar following an overnight fast, but were decreased in the HIRECO after glucose challenge. HIRECO mice demonstrated significant endothelial dysfunction measured by a blunted response to acetylcholine (Emax, WT vs HIRECO: 84±3% vs 68±3% respectively; n=5, p>0.05). Endothelium-independent response to sodium nitroprusside remained unchanged. The impaired aortic response to acetylcholine was normalised by the specific NADPH oxidase inhibitor peptide, gp91ds-tat, (Emax: 93±5%; n=6, p<0.05), as well as the superoxide dismutase mimetic, Mn(III) tetrakis (1-methyl-4-pyrydil) porphyrin pentachloride. Isolated aortic rings of HIRECO exhibited a hypercontractile response to phenylephrine compared to wild type mice (log EC50, WT vs HIRECO: 6.96±0.03 vs 7.24±0.08, n=6, p<0.01). Indeed, HIRECO mice elicited a 1.65-fold increase in the level of superoxide anion production compared to WT. Basal NO bioactivity was decreased in HIRECO compared to WT littermates (Emax upon exposure to eNOS inhibitor, L-NAME in phenylephrine-constricted aorta, WT vs HIRECO: 144±27.9% vs 32±33%, n=5, p<0.05). However, basal eNOS phosphorylation levels in isolated endothelial cells of HIRECO mice was enhanced 1.56-fold compared to WT littermates.

Conclusions/Implications These data demonstrate enhanced oxidative stress in a novel murine model of increased insulin signalling in the endothelium, leading to reduced bioavailability of nitric oxide and atherosclerosis. These data also demonstrate for the first time, that increased insulin sensitivity in the endothelium, increases the generation of superoxide anion and reduces NO bioavailability.

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105 NOX2 NADPH—OXIDASE A NOVEL TARGET TO PREVENT INSULIN RESISTANCE RELATED ENDOTHELIAL CELL DYSFUNCTION

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Introduction Insulin resistance, a central pathophysiological feature of type 2 diabetes is characterised by a deleterious change in endothelial cell phenotype, a hallmark of which is increased generation of reactive oxygen species. We examined the role of NADPH oxidase and specifically NOX2 NADPH oxidase in insulin resistance induced endothelial cell dysfunction. We studied mice with endothelium specific over expression of a dominant negative insulin receptor (ESMIRO) and mice with whole body haploinsufficiency of the insulin receptor ($IR^{+/-}$).

Methods ESMIRO mice, a model of endothelium specific insulin resistance, and $IR^{+/-}$ mice a model of whole body insulin resistance were used to examine the effect of acute and chronic pharmacological inhibition of NADPH oxidase on superoxide production (lucigenin enhanced chemiluminescence) and endothelial function (acetylcholine mediated aortic relaxation). To specifically investigate the role of NOX2, we crossed mice with holoinsufficiency of NOX2 with ESMIRO mice to generate ESMIRO/$NOX2^{y/-}$ mice. Data expressed as mean±SEM; male mice used for all experiments.

Results Basal superoxide generation in isolated pulmonary endothelial cells (PEC) was increased in both models of insulin resistance (by 130% in ESMIRO and 220% in $IR^{+/-}$ compared to wild type, both p<0.01; n=3 for each group). Pre-treating PEC with gp91ds-tat, a cell permeable specific blocker of NOX subunit assembly and function, reduced the excessive superoxide generation in ESMIRO and $IR^{+/-}$. Endothelial NO mediated vasorelaxation in aortic rings from ESMIRO and $IR^{+/-}$ was impaired (101%±11% relaxation to 1 μM acetylcholine in wild type, 61%±3% in ESMIRO (n=5, p<0.01); 91%±3% relaxation in wild type, 75%±6% in $IR^{+/-}$ (n=4, p=0.03)). This was restored by pre-incubating rings with gp91ds-tat (92%±6% relaxation in ESMIRO and 93%±6% in $IR^{+/-}$). Chronic (4 weeks) administration of gp91-ds tat peptide (using osmotic mini-pump) to ESMIRO and $IR^{+/-}$ mice also restored endothelial dependent relaxation (from 83%±11% to 100%±9% in ESMIRO and to 136%±11% in $IR^{+/-}$). NOX2 gene expression was significantly higher in ESMIRO mice. ESMIRO/$NOX2^{y/-}$ mice with complete deletion of NOX2 had significantly greater relaxation responses to acetylcholine than ESMIRO (77%±6% relaxation in ESMIRO and 100%±4% in ESMIRO/$NOX2^{y/-}$; n=5, p=0.002). Neither pharmacological nor genetic inhibition of NADPH oxidase had any effect on glucose homeostasis.

Discussion These data in complementary models of insulin resistance demonstrate that acute or chronic pharmacological inhibition of NADPH oxidase reduces superoxide generation and improves endothelial function. Specifically targeting NOX2 also restored endothelial function in ESMIRO mice.

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106 ROLE OF NEURONAL VS ENDOTHELIAL NITRIC OXIDE SYNTHASE IN THE CORONARY BLOOD FLOW RESPONSE TO PACING

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Background Endothelial nitric oxide synthase (eNOS) has been assumed to be the major source of nitric oxide (NO) regulating human coronary blood flow (CBF). In recent first-in-human studies with a neuronal NOS (nNOS)-selective inhibitor, we provided evidence that nNOS-derived NO regulates basal coronary blood flow whereas eNOS mediates increases in flow in response to the endothelial agonist, substance P. The present study aimed to investigate the effects of nNOS vs eNOS inhibition on coronary blood flow response to increased heart rate.

Methods We studied the effects of the nNOS-selective inhibitor, S-methyl-L-thiocitrulline (SMTC), and the non-selective NOS inhibitor, NG-monomethyl-L-arginine (L-NMMA) at doses previously shown to inhibit nNOS or both nNOS and eNOS, respectively. 18 patients already undergoing elective cardiac catheterisation for clinical reasons and found to have normal coronary arteries were included. An intracoronary Doppler flow wire was positioned in the coronary artery for measurement of blood flow velocity whereas

coronary artery diameter was measured by quantitative angiography. An incremental pacing protocol that raised heart rate to a maximum of 150 bpm was undertaken in all patients via a temporary right atrial pacing wire. Pacing was performed in the presence of saline vehicle and then either L-NMMA or SMTC (one inhibitor per patient; n=9 each group).

Results SMTC (0.625 μmol/min) and L-NMMA (25 μmol/min) both reduced basal CBF to a similar extent (−22.8%±1.24% vs −26.8%±2.16%; n=9 each; p=NS). During saline infusion, CBF increased with atrial pacing from 58.7±9.90 to 87.4±17.3 ml/min (n=9, p<0.05). During L-NMMA, the increase in CBF was significantly blunted compared to that during saline (ΔCBF 17.7±4.54 ml/min vs 28.7±8.08 ml/min during saline; n=9, p<0.05 by 2-way ANOVA). In patients receiving SMTC, however, the increase in CBF with pacing was similar to that during saline (ΔCBF 36.5±6.05 ml/min vs 25.2±6.65 ml/min during saline; n=9, p=NS by 2-way ANOVA). SMTC and L-NMMA both reduced basal coronary artery diameter to a similar extent (n=9). L-NMMA blunted the pacing-induced increase in coronary artery diameter (n=9, p<0.05 vs saline vehicle) whereas SMTC had no effect (n=9, p=NS).

Conclusion These results suggest that increases in human coronary blood flow in response to incremental atrial pacing are mediated by eNOS-derived NO rather than nNOS-derived NO.

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107 REMOTE ISCHAEMIC PRECONDITIONING AND HUMAN ATRIAL TRABECULAE IN THE DIABETIC HEART

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Introduction Remote ischaemic preconditioning (RIPC) using brief cycles of upper or lower limb ischaemia and reperfusion has been reported to protect the heart against ischaemia-reperfusion injury (IRI). Previous studies suggest that the diabetic heart is more resistant to the cardioprotective effects of myocardial ischaemic preconditioning. Whether the diabetic heart is amenable to RIPC is unknown and is investigated in this study.

Methods Non-diabetic and diabetic patients undergoing elective coronary artery bypass graft (CABG) surgery were randomised to receive three different treatment protocols after the induction of anaesthesia: (1) Control—no RIPC; (2) RIPC1 comprising 3-five min cycles of upper arm cuff inflation/deflation; or (3) RIPC2 comprising 2-five min cycles of simultaneous upper and lower limb cuff inflations/deflations (total 4). A section of the right atrial appendage was harvested, from which atrial trabeculae were isolated and subjected to 90 min simulated ischaemia and 120 min simulated reperfusion, at the end of which the recovery of baseline contractile function was determined.

Results Atrial trabeculae harvested from diabetic (N=13 patients) and non-diabetic control patients (N=20 patients) were demonstrated to recover 24.5%±2.4% and 29.3%±1.3% of baseline contractile function, respectively. Prior treatment of patients with RIPC1 increased the recovery of function in both non-diabetic (50.4%±1.9%; p<0.05) and diabetic (41.6%±1.9%; p<0.05) patients. Interestingly, the stronger RIPC2 stimulus resulted in a greater recovery of function in both non-diabetic (59.3%±1.9%; p<0.05) and diabetic (50.7%±2.1%; p<0.05) patients. As a positive control direct hypoxic preconditioning (HPC) of atrial trabeculae also improved the recovery of function (56.4%±1.8% with HPC vs 27.5%±1.7% in control; N=10 patients; p<0.05). The administration of the MEK-Erk1/2 inhibitors U0126 and PD98059 at the onset of reperfusion abrogated the protective effect in both non-diabetic (30.9%±0.8% U0126 and 31.3%±0.8% PD98059; p>0.05) and diabetic (28.6%±0.9% U0126 and 30.0%±1.2% PD98059; p>0.05) atrial trabeculae.

Conclusion We demonstrate for the first time that in vivo RIPC can protect ex vivo atrial trabeculae against simulated IRI. Both non-diabetic and diabetic atrial trabeculae were amenable to RIPC protection. Increasing the intensity of the RIPC stimulus resulted in greater functional recovery. The pro-survival kinase MEK-Erk1/2 appears to contribute to RIPC protection in human atria trabeculae.

108 REMOTE ISCHAEMIC CONDITIONING IS IMPAIRED IN DIABETES

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Aim Remote conditioning, whereby intermittent non-critical ischaemia of remote peripheral muscle can protect cardiac muscle from ischaemia-reperfusion (I-R) injury even when applied after the onset of cardiac ischaemia, has emerged as a potential therapeutic manoeuvre for reducing I-R injury. Whether such protection is mediated via serum factors and whether the development of the protection is impaired in disease states is unclear. We sought to develop a cell based model for assessing I-R injury, and utilised this to screen sera from both control healthy volunteers and type 1 diabetic patients for blood borne cardioprotective signals capable of mediating remote conditioning protection.

Methods Subjects studied included healthy volunteers and patients with type 1 diabetes with microvascular complications (retinopathy, microalbuminuria). Control (unconditioned) blood samples were taken prior to remote conditioning. An upper limb was then occluded with a tourniquet [30 mm Hg suprasystolic, 5 min] and released to reperfuse the arm [5 min] three times. Conditioned blood was collected immediately afterwards from the contralateral arm. Serum was separated and stored at −80°C until assay. Serum was screened for its protective capacity using a cellular model of I-R. Myocardial ischaemia was simulated by centrifugation of freshly isolated rat cardiac ventricular myocytes into a pellet [30 min]. Gaseous diffusion was prevented by an impermeant layer of mineral oil. Reperfusion injury was simulated through dispersal of the pellet in oxygenated saline solution. Cell viability was determined by propidium iodide staining for necrosis and calcein-AM counterstaining for viability.

Results In healthy subjects (n=21) unconditioned serum resulted in necrosis of 23.7%±4.9% (mean±SEM) of cells. Conditioned serum resulted in a significant reduction of necrosis to 6.4%±5.1% (p<0.05). However conditioned serum from diabetics showed no evidence of protection compared with unconditioned diabetic serum (51.1%±4.6% vs 45.7%±6.4% respectively, n=14, p>0.05, NS). The difference in necrosis rates between conditioned serum from healthy subjects and diabetics was highly significant (p<0.001). Results were similar whether the serum was applied before centrifugation of the cells (mimicking preconditioning in vivo) or on reperfusion (mimicking postconditioning).

Conclusion We report the development of a novel bioassay that can be used to quantitatively assess the strength of the serum-based signal that mediates remote conditioning. We further show that this signal is significantly impaired in patients with advanced diabetes. The later finding has important implications for the interpretation of clinical trials of remote conditioning, which include diabetics.

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