Young research workers' prize finalists

A DYSSYNCHRONOUS ACTIVATION IN HETEROZYGOUS CX43 GERMLINE KNOCKOUT MICE INDUCES STEADY-STATE POTASSIUM CURRENT REMODELLING AND SUSTAINED DYSSYNCHRONY

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Right ventricular pacing induces dyssynchronous cardiac activation, an independent predictor of worsened outcomes, in patients with reduced systolic function. Dyssynchrony is associated with gap junction remodelling, a downregulation of connexin (Cx)43 expression and regional distribution. Gap junction remodelling is strongly associated with arrhythmias. We hypothesised that dyssynchronous activation in a mouse heart expressing 50% less Cx43 (with otherwise normal function) would result in aberrant cardiac performance and electrophysiology compared with wild-type (WT) littermates.

Right ventricular epicardial pacing for 6 h at 10% above the average anaesthetised sinus rate was used. We found no significant differences in baseline echocardiographic ventricular function in the WT or Cx43+/− subgroups. Cx43+/− mice at baseline demonstrated significant septal-to-posterior wall motion delay (SPWMD) compared with WT ($p<0.01$). After pacing or sham-pacing SPWMD in the WT (4.3 ± 0.7 ms and 6.2 ± 0.7 ms, respectively) and in sham-paced Cx43+/− mice (7.2 ± 1.5 ms) as well as other echocardiographic indices were unchanged. However, SPWMD, worsened significantly in paced Cx43+/− hearts (17.7 ± 2.3 ms; $p<0.01$). Two hours after cessation of pacing, SPWMD remained significantly elevated in the Cx43+/− mice (15.1 ± 0.2 ms; $p<0.01$) compared with baseline.

We optically mapped isolated Cx43+/− hearts. We found no significant delay in right-to-left epicardial breakthrough patterns during sinus rhythm pre and post-pacing (0.40 ± 0.32 ms and 0.62 ± 0.25 ms, respectively). This suggests that sustained dyssynchrony in Cx43+/− hearts does not result from an alteration of epicardial activation breakthrough pattern or differences in interventricular conduction.

The in-vivo ventricular effective refractory period (VERP; at a pacing cycle length of 100 ms) was unchanged after pacing or sham-pacing in WT (34.7 ± 2.8 ms and 34.3 ± 3.6 ms, respectively) or after sham-pacing in Cx43+/− mice (30.3 ± 1.8 ms). After pacing, however, the VERP100 in Cx43+/− mice prolonged significantly (49.4 ± 5.1 ms; $p<0.01$). Two hours after cessation of pacing, the VERP100 remained elevated in the paced Cx43+/− mice (50.0 ± 9.0 ms; $p<0.01$). No sustained arrhythmias were induced.

Whole cell patch recordings demonstrated significantly shorter action potential duration (APD) in non-paced Cx43+/− hearts compared with those from WT. Pacing was associated with significant prolongation of the APD in Cx43+/− myocytes. The steady-state potassium current (Iss) was significantly increased in non-paced Cx43+/− myocytes compared with WT, without corresponding alterations in Ito or Ik1. Pacing resulted in a significant diminution of Iss current density in Cx43+/− cardiomyocytes, probably accounting for reciprocal pacing-related changes in APD. In contrast, pacing had no effect on APD or Iss in myocytes isolated from WT.

Dyssynchronous activation and reduced Cx43 expression sensitises the myocardium to significant electrical remodelling possibly by a positive mechano-electric feedback loop.

B MECHANISMS OF CARDIAC PLEIOTROPY IN *CITED2* DEFICIENCY

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Cited2 gene deletion results in pleiotropic and penetrant cardiovascular malformations, laterality defects, adrenal agenesis, neural tube defects and fused cranial ganglia. Heterozygous *CITED2* mutations are associated with varied congenital heart disease in humans suggesting haploinsufficiency. *CITED2* acts as a transcriptional co-activator and as a transcriptional repressor of HIF1A. *Cited2* loss reduces levels of the left–right patterning genes *Nodal*, *Pitx2c* and *Lefty2* in the left lateral plate mesoderm, *Pitx2c*, in the heart and may increase levels of Vegfa, a HIF1A target. We investigated the cardiovascular pleiotropy in *Cited2* deficiency using a conditional knockout approach.

Methods: Mice with a *Cited2* flox allele were crossed with Cre-expressing mouse strains allowing conditional *Cited2* deletion. The *Cited2* flox allele activated lacZ expression following Cre recombination, allowing assessment of recombination efficiency by lacZ staining. Embryos were examined by magnetic resonance imaging and histology. Quantitative reverse transcriptase PCR was performed on embryonic day 13.5 heart RNA.

Results: Epiblast *Cited2* deletion using *Sox2Cre* completely recapitulated the global knockout phenotype with 10/10 knockout embryos showing cardiac defects and 6/10 left–right patterning defects. Neural crest deletion with *Wnt1Cre* resulted in cranial ganglia fusions and exencephaly but no laterality or cardiac abnormality. Mesodermal deletion using *Nkx2-5Cre*, *Mesp1Cre* or *Isl1Cre* resulted in varying penetrance of only septal defects (6/11, 2/18 and 0/14, respectively), staining suggesting recombination efficiencies over 90%. Cardiac *Cited2* levels were reduced 4.7-fold ($p = 1.1 \times 10^{-5}$) in *Cited2*−/flox;*Nkx2-5Cre* hearts compared with *Cited2*+/flox;*Nkx2-5Cre* controls but *Pitx2c* expression was unchanged. Vegfa was reduced 1.54-fold ($p = 0.018$) providing a molecular mechanism for the septal defect observed. Investigating potential mechanisms of haploinsufficiency in congenic *Cited2* heterozygote hearts revealed a significant reduction in *Cited2* and *Pitx2c* expression, the expression of both being highly positively correlated ($r = 0.68$, $p = 0.0009$). *Pitx2c* is critical for cardiac development and deficiency suggests a molecular mechanism linking left–right patterning genes to the septal defects observed in *Cited2* heterozygous embryos. No difference in Vegfa expression between wild-type and *Cited2* null hearts was seen, likely to reflect global embryonic hypoxia overriding the action of *Cited2* on Vegfa transcription.

Conclusions: This work indicates that defective left–right patterning in epiblast derivatives: (1) drives the pleiotropism observed in *Cited2* deficiency; (2) identifies Vegfa as a novel *Cited2* target gene and (3) shows that *Cited2* haploinsufficiency may result from *Pitx2c* deficiency. Diverse cardiac malformations can arise from a single gene defect and aberrant left–right patterning may cause congenital heart disease even in the absence of a classic laterality defect such as isomerism.

C DIFFERENCES IN CORONARY ARTERY HAEMODYNAMICS DUE TO CHANGES IN FLOW AND VASCULAR GEOMETRY AFTER PERCUTANEOUS CORONARY INTERVENTION

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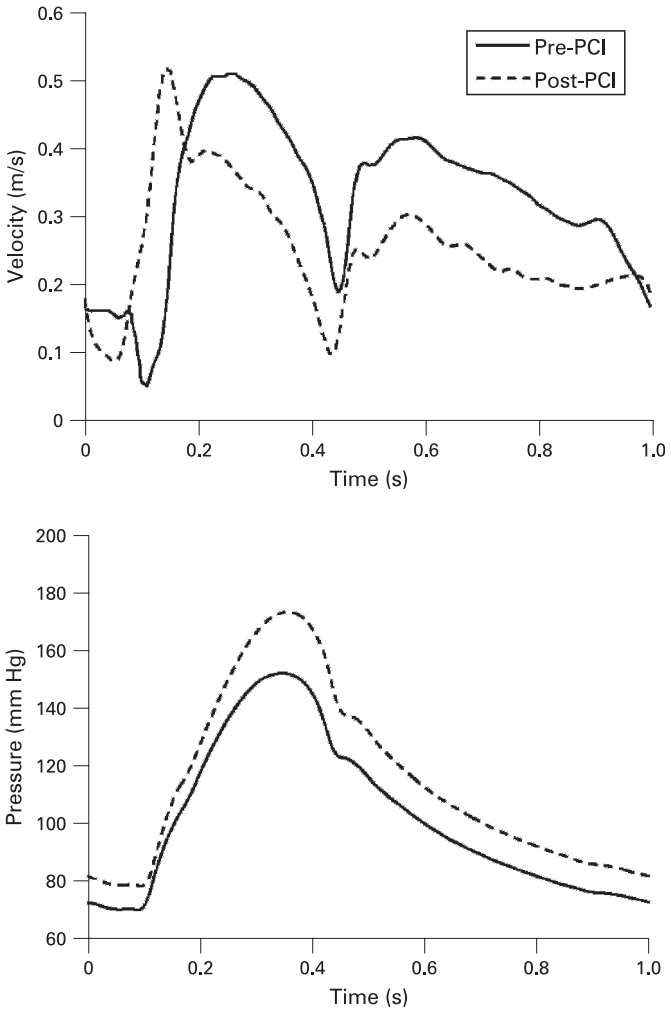
The right coronary artery (RCA) is a common site of atherosclerosis, which is linked to haemodynamic factors such as low and

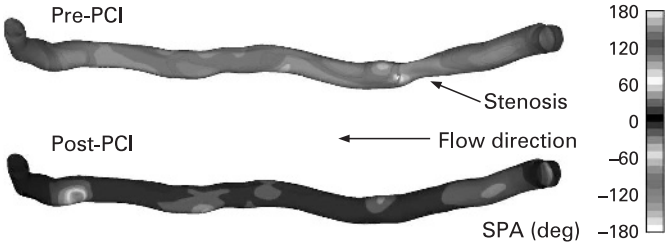


Abstract C Figure 1 Velocity (left) and pressure (right) waveforms pre and post-percutaneous coronary intervention (PCI).

oscillating wall shear stress (WSS). The effects of the difference in blood flow waveform and arterial geometry before and after percutaneous coronary intervention (PCI) on coronary haemodynamics in RCA were investigated using computational fluid dynamics (CFD).

An RCA from a patient with a 65% stenosis was reconstructed based on multislice computed tomography images acquired before PCI and a non-stenosed coronary artery was modelled based on the computed tomography-based RCA geometry. Blood flow in the RCA models was simulated using CFD with pulsatile inflow conditions based on flow and pressure waveforms acquired with an intravascular ultrasound Doppler probe in the RCA of a patient undergoing PCI.

The velocity waveform changed considerably after PCI as a result of the change in arterial impedance following PCI; velocity is higher for the post-PCI waveform particularly in the middle of the cardiac cycle. Pressure waveforms, however, remained similar after PCI although the amplitude of the pressure increased by approximately 15 mm Hg. The difference in the waveforms before and after PCI did not affect time-averaged WSS and oscillatory shear index, which are commonly used indices to assess the influence of haemodynamics on endothelium (fig 1). However, phase-lag between pressure and WSS on the endothelium (stress phase-angle; SPA), which regulates the secretion of vasoactive biomolecules (nitric oxide, prostacyclin and endothelin-1), was altered by the difference in the waveforms (fig 2). The range of SPA was −219 to −25.5° for the pre-PCI state but −86.6 to 9.93° for the post-PCI state; more



Abstract C Figure 2 Stress phase-angle (SPA) profile on the epicardial side of the right coronary artery for the pre and post-percutaneous coronary intervention (PCI) state.

asynchronous for the pre-PCI state. Asynchronous SPA (−180°) was reported to cause a pro-atherogenic pattern of vasoactivator production in comparison with synchronous SPA (0°). Highly asynchronous SPA occurs in the poststenotic region and areas of curved and irregularly angulated surface. Blood flow in post-PCI geometry with the pre-PCI waveform was also simulated to investigate the effects of the pre-PCI waveform separately. It was found that the pre-PCI waveform caused highly asynchronous SPA (approximately −130°) even without stenosis.

CFD is a useful technique for predicting potentially atherogenic regions when used in conjunction with flow and geometric information from individual patients. The physiological implication of the results is that differences in the pulsatile flow waveform are more likely to induce a pro-atherogenic haemodynamic condition in terms of stress phase-angle than a geometric change of an artery, although the difference in waveform has a relatively small impact on commonly used, and important, haemodynamic parameters, time-averaged WSS and oscillatory shear index.

D A NOVEL LOCUS—*PSRC1/CELSR2* GENE LOCUS ON CHROMOSOME 1P13.3, WHICH AFFECTS SERUM CHOLESTEROL AND IS ASSOCIATED WITH INCREASED RISK OF CORONARY ARTERY DISEASE

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Background: Through genome-wide association studies we have recently identified seven novel loci on 1p13.3 (*PSRC1/CELSR2* genes), 1q41 (*MIA3* gene), 2q36.3 (non-genic region), 6q25.1, (*MTHFD1L* gene), 9p21.3 (*CDKN2A* and *CDKN2B* genes), 10q11.21 (non-genic region), and 15q22.33 (*SMAD3* gene) that confer a substantial increase in the risk of coronary artery disease (CAD). Elucidating the mechanisms by which these loci affect CAD risk could have important clinical utility. Here we investigated whether these loci act through mechanisms involving traditional cardiovascular risk factors.

Methods and Results: We genotyped the lead single nucleotide polymorphisms in the seven CAD-associated loci in 2037 adult individuals from 520 nuclear families characterised for body mass index, waist–hip ratio, 24-h ambulatory blood pressure, total cholesterol, high-density lipoprotein cholesterol and glucose. Single nucleotide polymorphism rs599839, representing the locus in the vicinity of the *PSRC1* and *CELSR2* genes on chromosome 1p13.3 showed a strong association with total cholesterol. The CAD-associated risk allele A of rs599839 (allele frequency 0.78) was associated with a 0.17 mmol/l (95% CI 0.10 mmol/l to

0.24 mmol/l) higher serum cholesterol level per allele copy ($p = 3.84 \times 10^{-6}$). The association of rs599839 with total cholesterol was confirmed in an independent cohort (n = 847) of healthy adults ($p = 1.0 \times 10^{-4}$, combined $p = 1.7 \times 10^{-9}$) and shown to be related to an effect on low-density lipoprotein cholesterol ($p = 8.56 \times 10^{-5}$). None of the other variants showed a strong association with the measured cardiovascular risk factors.

Conclusions: The novel CAD-associated locus in the vicinity of the *PSRC1* and *CELSR2* genes on chromosome 1 enhances CAD risk through an effect on plasma low-density lipoprotein cholesterol. The findings provide a strong basis for further investigation of the role of these hitherto unsuspected genes in cholesterol metabolism and coronary risk.

E ENDOGENOUS XANTHINE OXIDASE CAUSES PROFOUND VASCULAR OXIDATIVE STRESS AND WIDESPREAD ENDOTHELIAL DYSFUNCTION IN CHRONIC STABLE ANGINA PATIENTS DESPITE CONTEMPORARY THERAPY

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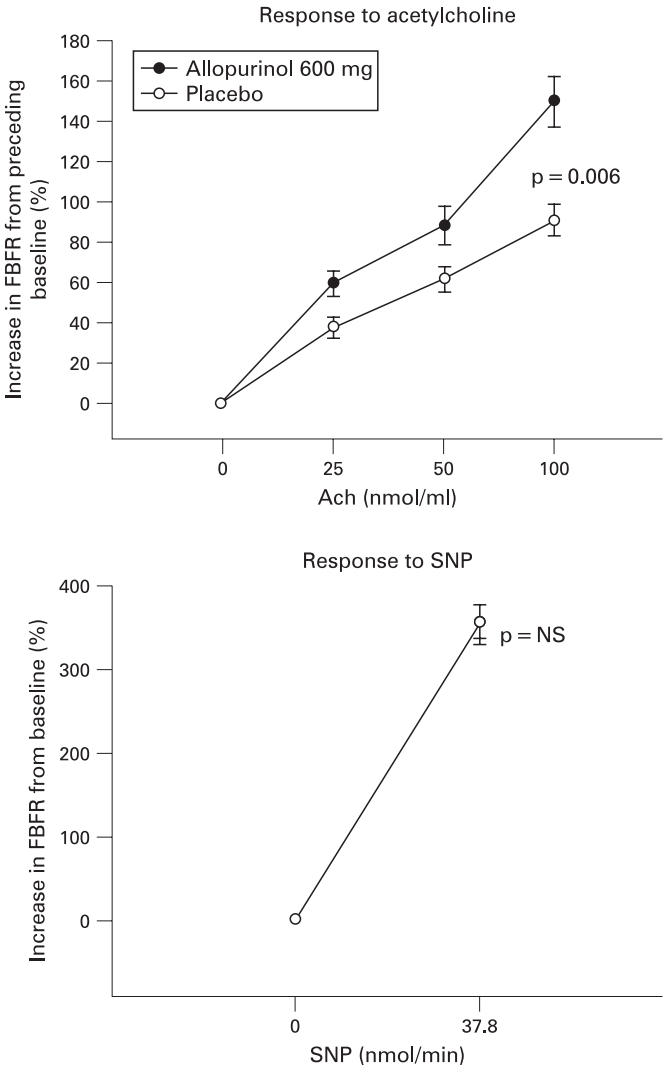
Introduction: To examine whether endogenous xanthine oxidase contributes to ongoing vascular oxidative stress and endothelial dysfunction in subjects with chronic stable angina on optimum medical therapy.

Methods and Results: A randomised double-blind, placebo-controlled, crossover study was conducted in 90 patients with angiographically documented coronary artery disease (CAD) and preserved left ventricular function (table 1). Subjects received 300 mg allopurinol for 4 weeks and 600 mg for the next 4 weeks or placebo for 8 weeks and subsequently crossed over after a 4-week washout. Endothelial function was assessed by forearm venous occlusion plethysmography, flow-mediated dilatation of brachial artery and central arterial stiffness was assessed by pulse wave analysis. There was a significant improvement in acetylcholine-mediated vasodilatation on allopurinol compared with placebo (145 ± 11% versus 93 ± 8%, p = 0.006). There was no significant difference in response to sodium nitroprusside (fig 1). Vascular oxidative stress was assessed using intra-arterial ascorbic acid and the increase in acetylcholine-induced vasodilatation in the presence of vitamin C was completely abolished by allopurinol pre-treatment (fig 2). Allopurinol also significantly improved brachial artery FMD (table 2), whereas placebo had no effect (4.2 ± 1.8% to 5.44 ± 1.7%, p<0.001 versus 4.2 ± 1.8% to 4.12 ± 1.8%, p = NS). There was no difference in response to glyceryl trinitrate between the two treatments. The central augmentation index improved significantly on allopurinol (27.32 ± 4.98% to 24.69 ± 4.55%; 95% CI 0.41 to 4.84, p<0.001, whereas there was no significant change with placebo (27.76 ± 5.93% to 27.63 ± 5.54%; 95% CI −2.4 to 2.64, p = 1.0).

Abstract E Table 1 Baseline characteristics

Total no of patients completing study (male/female)	80 (64/16)
Age (years)	65.5 ± 7.73
No of vessels diseased (1/2/3)	17/30/33
Med Rx/PCI/CABG	11/47/22
ASA/clopidogrel	76/4 (100%)
ACEI/ARB (%) (ramipril equivalent dose in mg)	58/10 (85%) (7.3)
Statins (%) (simvastatin equivalent dose in mg)	76 (95%) (37)
Creatinine clearance (ml/min)	78 ± 25
Systolic blood pressure (mm Hg)	132 ± 12
Diatolic blood pressure (mm Hg)	75 ± 8

ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; CABG, coronary artery bypass grafting; Med Rx, medical treatment; PCI, percutaneous coronary intervention.



Abstract E Figure 1
Ach, acetylcholine; FBFR, forearm blood flow; SNP, sodium nitroprusside.

Conclusion: Our study demonstrates that despite contemporary, evidence-based treatment for CAD endogenous xanthine oxidase continues to generate vascular oxidative stress and widespread endothelial dysfunction. The prospect arises that high dose allopurinol might reduce future atherothrombotic events in CAD over and above the current therapies.

F BASAL VASCULAR TONE IN HUMANS IN VIVO IS REGULATED BY NEURONAL NITRIC OXIDE SYNTHASE

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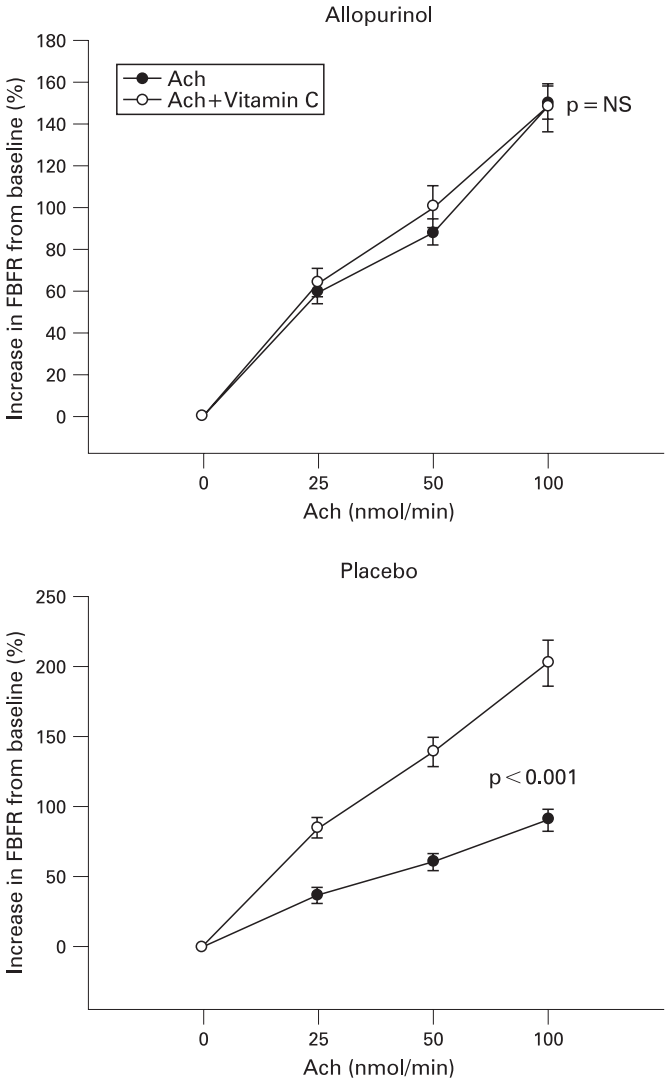
Introduction: Nitric oxide (NO) is an important regulator of human vascular tone, with dysfunctional release contributing to vascular disease. These effects have been attributed to NO produced by endothelial nitric oxide synthase (eNOS). Recent experimental data indicate that neuronal nitric oxide synthase (nNOS) may also be expressed in vessels and perivascular nerves, but its importance in human vascular regulation is unknown.

Methods and Results: We undertook a first-in-man study of the in vivo effects of an nNOS-selective inhibitor, *S*-methyl-L-thiocitrulline



Abstract E Figure 2
Ach, acetylcholine; FBFR, forearm blood flow; SNP, sodium nitroprusside.

(SMTC). Local brachial artery infusion of SMTC (0.025–0.2 μmol/min) in healthy subjects caused a dose-dependent reduction in plethysmographically measured basal forearm blood flow, with a 30.1 ± 3.8% decrease at the highest dose (n = 10; mean ± SE; $p<0.01$). This effect was abolished by co-infusion of the NOS substrate L-arginine but was unaffected by D-arginine. A similar reduction in basal flow with the non-selective NOS inhibitor, N^Gmonomethyl-L-arginine (L-NMMA: 37.4 ± 3.1%, n = 10; $p<0.01$) required a 20-fold higher dose of 4 μmol/min. Acetylcholine-induced vasodilatation (considered to be eNOS-mediated) was significantly reduced (>60%; $p<0.01$) by L-NMMA 4 μmol/min but was unaffected by SMTC (p = ns). The vasodilator response to sodium nitroprusside was unaltered by SMTC. To investigate the effects of SMTC in a different vascular bed, we examined the effect of intracoronary infusion in patients with angiographically normal coronary arteries undergoing cardiac catheterisation. SMTC (0.625 μmol/min) reduced basal coronary flow (Doppler flowire) by 40 ± 7.0% (n = 6; $p<0.05$) but had no significant effect on vasodilation to intracoronary substance P (20 pmol/min). In contrast, intracoronary L-NMMA (25 μmol/min) reduced coronary flow by 23 ± 7.2% and inhibited the substance P response by 60 ± 4.0% (n = 6; $p<0.01$). To assess whether SMTC effects resulted from inhibition of nNOS in the vessel wall, isolated human internal mammary artery rings from coronary artery bypass graft patients were studied. Neither SMTC (10 μmol) nor L-NMMA altered basal tone but L-NMMA abolished acetylcholine-induced relaxation whereas SMTC had no effect (n = 6; p = ns). To assess whether SMTC might affect nNOS in local nerves, mental stress-stimulated increases in forearm blood flow were studied. These were significantly inhibited by SMTC 0.2 μmol/min (54 ± 7.6%, n = 10, $p<0.01$) and similarly by L-NMMA 2 μmol/min (62 ± 9.7%, n = 10, $p<0.005$).

Conclusion: These data suggest that basal blood flow in both the human forearm and coronary vascular beds is regulated by local nNOS-derived NO, whereas acetylcholine-stimulated or substance P-stimulated vasodilatation may be eNOS-mediated. The cellular source of nNOS that is involved may be perivascular nerves rather than the vessel wall. Thus, nNOS and eNOS play distinct local roles in the physiological regulation of human vascular tone *in vivo*.

Funding: This study was supported by the British Heart Foundation.

Abstract E Table 2

	Baseline	Post-placebo	95% CI p value	Baseline	Post-allopurinol	95% CI p value
FMD (%)	4.2 ± 1.8	4.12 ± 1.8	−1.12 to 1.08 p = 0.76	4.2 ± 1.7	5.44 ± 1.8	0.18 to 1.42 p<0.001
GTN (%)	14.1 ± 6.0	15.0 ± 6.0	−1.9 to 0.12 p = 0.08	14.6 ± 5.8	14.2 ± 5.7	−0.26 to 1.0 p = 0.23
AIx (HR 75) (%)	27.76 ± 5.93	27.63 ± 5.54	−2.4 to 2.64 p = 1.0	27.32 ± 4.98	24.69 ± 4.55	0.41 to 4.84 p<0.001

AIx, augmentation index; FMD, flow-mediated dilatation; GTN, glyceryl trinitrate.