

Abstracts



Report on the Spring Meeting of the British Atherosclerosis Society  
Medical Sciences Teaching Centre/Magdalen College,  
Oxford March 30–31, 2006

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Symposium on: The Metabolic Syndrome, organised by Philippa Talmud and Naveed Sattar.

Speakers and topics included:

The metabolic syndrome: origins and insights

Overview

Metabolic syndrome: what is its clinical value?

Origins of dyslipidaemia in the metabolic syndrome

Professor Sir George Alberti

Professor James Meigs

Professor Chris Packard

Prevention of the metabolic syndrome

Lifestyle change

Muscle physiology, skeletal muscle as an endocrine organ: role in preventing metabolic syndrome

Pharmacology

Professor Jaakko Tuomilehto

Professor Bengt Saltin

Professor Anthony Barnett

Genetics of the metabolic syndrome

Genetic epidemiology of the metabolic syndrome

Genes, nuclear receptors and the metabolic syndrome

Professor Philippe Froguel

Professor Krish Chatterjee

The John French Memorial Lecture was delivered by Professor Naveed Sattar, and entitled ‘Dangerous Liaisons: Metabolic and Vascular Risk Pathways’.

**Abstracts of the 10 free communications presented are given below:**

The next meeting of the Society will take place on 21–22 September 2006 at Queens College, Cambridge. This will be a joint meeting with the BSCR on Biomedical Signalling in Atherosclerosis, organised by Dorian Haskard, Peter Weinberg and Qingbo Xu.

Further details about this meeting are available from the BAS Secretary, Dr. Chris Newman, Cardiovascular Research Group, Division of Clinical Sciences (North), University of Sheffield, Sheffield S5 7AU, UK, (e-mail: [c.newman@sheffield.ac.uk](mailto:c.newman@sheffield.ac.uk)) website: [www.britathsoc.ac.uk](http://www.britathsoc.ac.uk).



## A POTENTIAL ROLE FOR GALECTIN-3 IN ATHEROSCLEROTIC PLAQUE PROGRESSION THROUGH MONOCYTE CHEMOATTRACTION AND MACROPHAGE ACTIVATION

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Galectins are a family of lectins that are involved in inflammation, cell adhesion, apoptosis and chemotaxis, and also function as scavenger receptors.

In a whole-transcriptome scan of atherosclerotic plaques obtained from carotid endarterectomy, four members of the galectin family were found to be upregulated in unstable regions of the plaque (−2, −3, −8, −9). Gal-3 mRNA was approximately 10 times more abundant than the other galectins.

Correlation of Gal-3 mRNA expression with the expression of cell markers ( $n=12$ ), together with immunohistochemical analysis of carotid plaques showed that Gal-3 is predominantly expressed by macrophages. Western blot analysis showed that there is a membrane bound and a secreted form of Gal-3 in the atherosclerotic plaque ( $n=9$ ). Only the secreted isoform was upregulated in unstable regions of the plaque. Soluble Gal-3 mediates monocyte chemoattraction in vitro and causes an up to 10-fold increase in macrophage expression of pro-inflammatory mediators, such as TNF $\alpha$  and RANTES in a dose-dependent manner.

In conclusion, Gal-3 represents a new class of inflammatory mediators, which may contribute to atherosclerotic plaque progression through monocyte chemoattraction and macrophage activation. Gal-3 could be a novel target for anti-inflammatory drugs in the treatment of atherosclerosis.

## CRP IN THE PREDICTION AND PATHOGENESIS OF CARDIOVASCULAR EVENTS IN MIDDLE-AGED MEN; RESULTS FROM THE NPHS II STUDY

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The CRP-coronary heart disease (CHD) association may have prognostic utility or indicate a causal role in atherosclerosis, through blood pressure [BP], glycaemic status, or coagulant pathways. However, predictive utility above traditional risk factor assessment may be limited, and the association may be the result of confounding or reverse causation.

**Methods:** In the NPHS II study (3052 men; 10-year follow up; 227 CHD events), we evaluated the performance of CRP as a screening test for CHD, compared this with traditional risk factors and used Mendelian randomisation to test if associations between CRP, atherogenic phenotype and CHD are likely to be causal.

**Results:** CRP was associated with incident CHD (hazard ratio: 2.61; 95%CI: 1.78–3.82; tertile 3 versus 1), but also with age, BMI, smoking, lipids, BP, the Framingham score (FRS), and fibrinogen ( $p<0.001$  for all). CRP alone performed poorly as a screening test for CHD (area under ROC curve [AUC]: 0.61, 95%CI: 0.57–0.66), and its addition to the FRS did not substantially increase performance (AUC 0.64, 95%CI: 0.6–0.69). SNPs in the CRP gene (three tagging, one functional) were associated with CRP concentration (differences between homozygous subjects: 0.68–0.73 mg/L,  $p<0.001$ ), as were the haplotypes derived from them. However, none showed association with any other phenotype nor with risk of CHD.

**Conclusions:** CRP itself is associated with risk phenotypes and CHD risk, but CRP SNPs (and haplotypes) influencing its concentration showed no association. CRP is poorly predictive of CHD, and associations of CRP with risk phenotypes and CHD may be subject to residual confounding and to reverse causation.

## DISCOVERY OF VASOCONSTRICTOR ACTION OF KISSPEPTINS IN HUMAN CARDIOVASCULAR SYSTEM

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**Objective:** The orphan G-protein coupled receptor KISS1 (GPR54) has been paired with products of the Kiss-1 metastasis suppressor gene, kisspeptin (KP)-54, KP-14, KP-13 and KP-10. KPs are inhibitors of MMP-2 and MMP-9, which are increased in vulnerable regions of atherosclerotic plaques. Our aim was to determine the role of KPs in human vasculature.

**Methods and results:** RT-PCR showed discrete localisation of KISS1 to smooth muscle (SM) of developmentally related human tissues umbilical vein (UV), aorta and coronary artery (CA), the latter of which are prone to atherosclerotic plaque formation. Fluorescence dual labelling immunocytochemistry detected co-localisation of KISS1 and KPs to atherosclerotic plaques of CA and aorta and of KPs to vascular endothelial cells. Specific binding of [<sup>125</sup>I]KP-13 was detected in SM of aorta ( $K_D$   $0.37 \pm 0.17$  nM,  $B_{max}$   $6.2 \pm 0.6$  fmol/mg protein). In vitro studies on isolated rings of human CA ( $n=3$ ) and UV ( $n=3$ ) identified a potent vasoconstrictor action of KP-54 in these tissues ( $pD_2 \pm S.E.M.$   $8.89 \pm 1.57$ ,  $9.33 \pm 0.96$ ;  $E_{max} \pm S.E.M.$   $26.1 \pm 7.8$ ,  $45.5 \pm 2.9\%$  KCl, respectively).

**Conclusion:** KISS1 and KPs are expressed in atherosclerotic plaques of human aorta and CA. Furthermore KPs are potent constrictors of human CA and UV. This discovery suggests a previously undescribed role for KPs in cardiovascular disease.

### DISSOCIATION OF PHENOTYPIC AND FUNCTIONAL ENDOTHELIAL PROGENITOR CELLS IN PATIENTS UNDERGOING CORONARY INTERVENTION

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**Background:** Endothelial progenitor cells (EPCs) are circulating mononuclear cells with the capacity to mature into endothelial cells, and to contribute to vascular repair. We assessed the effect of local vascular injury during percutaneous coronary intervention (PCI) on circulating EPCs in patients with stable coronary heart disease.

**Methods:** EPCs were quantified by whole blood flow cytometry (CD34<sup>+</sup>KDR<sup>+</sup> phenotype), complemented by real-time PCR, and the colony forming unit (CFU-EPC) functional assay, before and during the first 24 h after diagnostic angiography ( $n = 20$ ) or PCI ( $n = 20$ ).

**Results:** Diagnostic angiography did not induce systemic inflammation or myocyte necrosis, nor affect the number of circulating CD34<sup>+</sup>KDR<sup>+</sup> cells or CFU-EPCs. PCI resulted in an increase in whole blood neutrophils ( $\Delta 1.31 \pm 0.35 \times 10^9/L$ ;  $p < 0.001$ ) and serum C-reactive protein concentrations ( $\Delta 2.5 \pm 1.5 \text{ mg/L}$ ;  $p = 0.001$ ), without significant myocardial necrosis. Twenty-four hours after PCI, the number and cellularity of CFU-EPCs increased ( $0.6 \pm 0.2$  versus  $2.3 \pm 0.9 \times 10^3$ ;  $p = 0.05$ ), although circulating CD34<sup>+</sup>KDR<sup>+</sup> cells ( $0.083 \pm 0.011$  versus  $0.083 \pm 0.010\%$  of mononuclear cells;  $p = 0.75$ ) and leucocyte CD34 mRNA (relative quantity  $2.26 \pm 0.48$  versus  $2.10 \pm 0.42$ ;  $p = 0.21$ ) did not. There was no correlation between CFU-EPCs and CD34<sup>+</sup>KDR<sup>+</sup> cells.

**Conclusions:** Local vascular injury following PCI results in a systemic inflammatory response and increases functional CFU-EPCs. This increase was not associated with an early mobilisation of CD34<sup>+</sup>KDR<sup>+</sup> cells, suggesting these cells are not the primary source of circulating EPCs involved in the immediate response to vascular injury.

### GENOME-WIDE MAPPING OF SUSCEPTIBILITY TO CORONARY ARTERY DISEASE IDENTIFIES A NOVEL REPLICATED LOCUS ON CHROMOSOME 17

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Coronary artery disease (CAD) has a complex, multifactorial etiology that includes a substantial heritable component. Identification of new genes involved in CAD may inform pathogenesis and provide new therapeutic targets. The PRO-CARDIS study recruited 2658 affected sib-pairs (ASPs) with onset of CAD before age 66 years from four European countries to map susceptibility loci for CAD. ASPs were defined as having CAD (CAD phenotype) if both had CAD, or myocardial infarction (MI phenotype) if both had a MI. In an initial study, involving a genome-wide linkage screen, tentative loci were mapped to chromosomes 3 and 11 with the CAD phenotype (1464 ASPs) and to chromosome 17 with the MI phenotype (739 ASPs). In a replication study, these loci were examined with a dense panel of grid-tightening markers in an independent set of families (1194 CAD and 344 MI ASPs). This showed a significant result on chromosome 17 (MI phenotype;  $p = 0.018$  after adjustment for three replication tests). An exclusion analysis suggests that further genes of effect size  $\lambda_{\text{sib}} > 1.24$  are unlikely to exist in these populations of European ancestry. In conclusion, this is the first genome-wide linkage analysis to both map and replicate a CAD locus. The region on chromosome 17 provides a compelling target within which to identify novel genes underlying CAD, with a view to developing novel preventative and/or therapeutic strategies.

### IGFBP-1 PROTECTS AGAINST THE METABOLIC AND VASCULAR CONSEQUENCES OF DIETARY-INDUCED OBESITY

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Insulin, insulin-like growth factor-I (IGF-I) and IGF binding protein-1 (IGFBP-1) have complimentary roles in the regu-

lation of glucose homeostasis. Observational studies suggest patients with insulin resistance and cardiovascular disease have reduced levels of IGF-I and IGFBP-1. We characterised longitudinal changes in the insulin/IGF-I/IGFBP-1 axis in mice fed an obesogenic diet and have demonstrated that the detrimental metabolic and vascular sequelae of dietary-induced obesity are attenuated in animals overexpressing human IGFBP-1. Wildtype mice (WT) receiving a high-fat diet had a higher body weight, larger fat pad mass and fat cell size and higher systolic blood pressure ( $p < 0.02$ ) than those fed chow diet. They demonstrated significantly decreased insulin sensitivity, measured by insulin tolerance testing (ITT). In addition the hypoglycaemic effect of IGF-I was decreased ( $p < 0.001$ ) and circulating IGF-I levels were found to be higher (298 ng/mL versus 404 ng/mL,  $p < 0.05$ ). Vascular insulin and IGF-I sensitivities as assessed by ex vivo in organ bath studies on thoracic aortic rings demonstrated no alteration on the effect of phenylephrine. Transgenic mice (TG) had similar body weights and fat pad depots compared to WT, however in contrast they showed preserved insulin sensitivity on ITT (41.8% decrease in blood glucose at 30 min versus WT 32.3%,  $p < 0.02$ ), normal blood pressure and significant blunting of vasoconstriction (pEC50  $1.47 \times 10^{-7}$  M versus  $2.87 \times 10^{-7}$  M for insulin and  $1.4 \times 10^{-7}$  M versus  $2.38 \times 10^{-7}$  M for IGF-I,  $p < 0.05$ ) in keeping with preserved vascular insulin and IGF-I sensitivities. These results suggest that IGFBP-1 protects against the development of insulin resistance in both peripheral tissues and the vasculature despite the development of obesity.

This work was supported by the British Heart Foundation.

### **INSULIN RESISTANCE CAUSES PREMATURE ENDOTHELIAL DYSFUNCTION THROUGH INCREASED PRODUCTION OF REACTIVE OXYGEN SPECIES**

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Cardiovascular disease accounts for 70% of deaths in diabetics and is often already established on presentation. The mechanism underlying this is unclear. We studied endothelial function in mice heterozygous for a knockout of the insulin receptor (IRKO) – a model of mild metabolic insulin resistance – and their wild type (WT) littermates, at 2 and 6 months of age. We aimed to identify early mechanisms of accelerated vascular dysfunction. Data presented as mean  $\pm$  S.E.M.,  $p < 0.05$  as significant. Studies of thoracic aorta in an organ bath apparatus confirmed normal vascular responses to phenylephrine, acetylcholine (ACH) and sodium nitroprusside at 2 months of age. By 6 months, IRKO mice developed impaired relaxation to ACH cf. WT ( $E_{\max}$   $66 \pm 5\%$  and  $87 \pm 4\%$ ). IRKO ACH responses were normalised following incubation with the

SOD mimetic MnTMPyP ( $E_{\max}$   $85 \pm 5\%$ ). Older IRKO mice were mildly hypertensive compared to WT ( $111 \pm 3$  and  $102 \pm 3$ ). Dihydroethidium (DHE) staining of aortic sections and FACS analysis of endothelial cells exposed to DHE confirmed increased superoxide production in IRKO mice. NADPH dependent superoxide production in IRKO endothelial cells (assessed by lucigenin enhanced chemiluminescence) was prevented by the Flavoprotein inhibitor DPI but not L-NAME.

We conclude that endothelial production of reactive oxygen species mediates premature endothelial dysfunction in insulin resistant states and represents a novel target for future drug therapies.

### **5-METHYL-TETRAHYDROFOLATE INCREASES NITRIC OXIDE BIOAVAILABILITY AND DECREASES SUPEROXIDE PRODUCTION BY IMPROVING eNOS COUPLING AND PREVENTING THE INTRACELLULAR OXIDATION OF TETRAHYDROBIOPTERIN, IN HUMAN VESSELS IN VIVO**

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*Background:* Uncoupled endothelial nitric oxide synthase (eNOS) is a source of superoxide radicals (SOO) in atherosclerosis. We hypothesized that 5-methyl-tetrahydrofolate (5MTHF), the active metabolite of folic acid, improves eNOS coupling by preventing intracellular oxidation of the eNOS cofactor tetrahydrobiopterin (BH4).

*Methods:* Fifty-six patients undergoing CABG were randomized to receive iv 5MTHF (0.13 mg/kg BW,  $n = 24$ ) or placebo ( $n = 32$ ) prior to graft harvesting. SOO was determined in paired samples of saphenous vein (SV) & mammary artery (MA) using lucigenin chemiluminescence, in the presence or absence of the NOS inhibitor LNAME. Vasomotor responses to acetylcholine (Ach) were determined by organ bath. Vascular 5MTHF and biopterin levels were measured by HPLC.

*Results:* 5MTHF improved vasomotor response to Ach & decreased SOO production in SV and MA ( $1.0 \pm 0.15$  and  $0.97 \pm 0.15$  RLU/s/mg in 5MTHF-treated versus  $2.27 \pm 0.37$  and  $5.01 \pm 0.62$  RLU/s/mg in placebo treated patients,  $p < 0.01$  for both). LNAME reduced the SOO signal in both HSV and IMA in placebo group, an effect reversed by 5MTHF. 5MTHF increased total biopterins (tBio), BH4 and the tBio/BH4 ratio. Vascular 5MTHF was correlated with BH4/tBio ratio ( $r = 0.406$ ,  $p = 0.001$  in HSV and  $r = 0.334$   $p = 0.043$  in IMA respectively).

*Conclusions:* 5MTHF increases nitric oxide bioavailability, improves eNOS 'coupling' and decreases SOO in human vessels, by preventing intracellular BH4 oxidation in vivo.

## THE ASSOCIATION OF UCP2-866G>A WITH PROSPECTIVE RISK OF TYPE 2 DIABETES IS DUE TO REDUCED INSULIN SECRETION FROM THE PANCREAS

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The key role of UCP2 in insulin secretion and energy balance makes it an attractive candidate for being important in the development of obesity and diabetes. The *UCP2*-866G>A is a functional variant with the -886A associated with higher mRNA in pancreatic B-cells. We determined the impact of this variant on the prospective risk of developing type 2 diabetes over 10 years in a cohort of 2936 healthy middle aged men. Homozygosity for the *UCP2A* allele increased the 10 years risk of diabetes by 1.94 times (HR [95%CI: 1.18–3.19];  $p=0.009$ ) compared to the GA+GG genotype. The genotype effect was additive with obesity, *UCP2AA* men with BMI > 30 kg/m<sup>2</sup> had a 10 years diabetes risk of 5.55 ([2.55–10.15];  $p<0.0001$ ). Consistent with this, we demonstrated 25% lower insulin secretion in the AA versus GG+GA ( $p=0.02$ ) healthy North European subjects in the HIFMECH study. As expected given its role in energy balance, AA subjects also had lower adiposity with a lower waist hip ratio (0.98+0.07 versus 1.0+0.06;  $p=0.03$ ). This lower fat mass is not sufficient to compensate for reduced insulin secretion in AA subjects who have higher prospective type 2 diabetes risk as a consequence. The risk is exacerbated when insulin demand is challenged further by the “environmental stress” obesity.

## VASCULAR EFFECTS OF PAR-1 AGONISM IN VIVO IN MAN

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**Background:** Protease-activated receptor type 1 (PAR-1) is a G-protein coupled receptor that mediates most of the cellular actions of thrombin. The aim of the study was to describe the vascular actions of PAR-1 agonism in man.

**Methods:** Dorsal hand vein diameter was assessed by the Aellig technique ( $n=14$ ) during infusion of the PAR-1 agonist SFLLRN (0.05–15 nmol/min), with and without norepinephrine (1–128 ng/min) or the glycoprotein IIb/IIIa antagonist tirofiban (250 ng/min). Forearm blood flow was assessed by venous occlusion plethysmography. In the presence of tirofiban (1250 ng/min), intrabrachial SFLLRN (5–50 nmol/min) and bradykinin (100–1000 pmol/min) were infused in 8 healthy volunteers. Blood was sampled to measure tissue-plasminogen activator (t-PA) release and platelet monocyte binding.

**Results:** SFLLRN caused vasoconstriction (from 100% to  $6 \pm 3\%$ ;  $p<0.001$ ) that was unaffected by norepinephrine or tirofiban co-infusion ( $p=ns$ ). In the arterial circulation, SFLLRN caused dose-dependent increase in forearm blood flow (from  $3.1 \pm 0.3$  to  $11.9 \pm 1.5$  mL/100 forearm;  $p<0.001$ ), t-PA release ( $8.1 \pm 1.4$  to  $12.7 \pm 1.5$  ng/mL;  $p=0.02$ ) and platelet monocyte binding ( $16 \pm 5\%$  to  $74 \pm 5\%$ ;  $p<0.0001$ ).

**Conclusions:** We have, for the first time, demonstrated that PAR-1 agonism in vivo causes platelet activation, vasoconstriction, vasodilatation and t-PA release. These unique and contrasting effects provide important insights into the pathophysiological role of thrombin in the human circulation.