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Contents

Editorial

Welcome to the January 2004 issue of *The* Bulletin and Happy New Year to everyone!

Our review article for this issue has been written by Mr. Jason Lee Johnson from the Division of Cardiovascular, Anaesthetic, and Radiological Sciences, University of Bristol Lessons focussing on "Atherosclerotic Plaque Rupture from the Apolipoprotein E-Deficient Mouse".

We are pleased to include a report on the recent BSCR meeting held at the University of Edinburgh entitled "*Oxidative Stress: from Measurement to Management*". The report has been written by the organiser, Gillian Gray and the abstracts presented at the meeting are also included. On behalf of the Society we wish to express our gratitude to the organisers for arranging an extremely successful and enjoyable meeting.

We can look forward to the BSCR meeting in Manchester this year in April. The Spring meeting on "*Frontiers in Cardiovascular Signalling*" is organised by David Eisner and Cathy Holt. A detailed programme for the meeting is included in this issue.

Finally, we bring you the latest details of grants awarded to researchers in the Cardiovascular field, by the Wellcome Trust.

Helen Maddock and Nicola Smart

Lessons in Atherosclerotic Plaque Rupture from the Apolipoprotein E-Deficient Mouse

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Atherosclerosis

The rupture of an atherosclerotic plaque with subsequent thrombotic lumenal occlusion is a primary cause of acute coronary events, accounting for 60% of sudden deaths [1]. The present dogma proposes that inflammatory cells play a major role in the pathophysiology of both atherosclerotic plaque development and rupture. Entrapment of low-density lipoprotein (LDL) within the extracellular matrix (ECM) of the vessel wall results in its modification and oxidation, which instigates an inflammatory response [2]. Leukocytes interact with numerous adhesion molecules, and various cytokines and growth factors secreted from endothelial cells and underlying vascular smooth muscle cells (VSMC), prompting their adhesion, transmigration and maturation (as reviewed by [3]). This inflammatory infiltrate triggers a series of events resulting in the formation of a complex atherosclerotic plaque. Such lesions contain a lipid/necrotic core that is highly thrombogenic but is protected from the circulating blood by the VSMC-rich, fibrillar collagen-rich fibrous cap which confers mechanical strength [4]. Proteases, including the matrix metalloproteinases (MMPs), are capable of degrading the collagen fibrils within the cap, thus reducing its strength and making it more prone to rupture [5]. VSMC apoptosis is also prevalent in the caps of unstable plaques [6]. Therefore, unchecked VSMC death jeopardises the structural integrity of the cap due to the reduction in VSMC-dependent collagen synthesis. In addition, inflammatory cells release a myriad of cytokines and growth factors capable of reducing VSMC collagen synthesis, inducing increased MMP expression and activation and further inflammatory cell recruitment (as reviewed by [3]).

Large animal models of atherosclerosis

Atherosclerotic plaque rupture is difficult to study in humans. Tissue samples retrieved at post mortem, or after endarterectomy or atherectomy, have allowed analysis of the events that occur pre- and post-rupture, but not the study of factors directly involved in acute rupture. Scientists have therefore attempted to develop animal models that mimic atherosclerotic plaque rupture, to allow direct interrogation of the mechanisms involved in this complex pathology. Furthermore, this would aid the design and testing of therapeutic interventions.

Atherosclerotic lesions have been induced in primates, pigs and rabbits fed diets containing a high percentage of cholesterol and fat [7]. Early lesions in these hyperlipidaemic/hypercholesterolaemic models mirror the fatty streaks observed in man. However, with time in pigs [8], and with intermittent cholesterol feeding in rabbits [9], more complex lesions exhibiting characteristics of advanced human atherosclerotic lesions form. Although myocardial infarction has recently been demonstrated in familial hypercholesterolemic rabbits [10], plaque rupture is not observed in this or in the pig model, demonstrating the lack of spontaneous rupture events and thrombosis in the above models.

Two other animal models have been reported where plaque rupture and thrombosis are induced by chemical or mechanical means. The first is the cholesterol-fed rabbit injected parenterally with the procoagulant Russell viper venom, and the vasoconstrictor histamine, which results in acute plaque disruption in 30% of animals [11]. This model was recently modified, by performing balloon injury to the rabbit aorta to accelerate lesion development [9]. The

second model is the balloon-injured cholesterol-fed rabbit whereby a balloon catheter is implanted in the thoracic aorta [12]. The balloon is inflated after an atherosclerotic lesion has formed around it, disrupting the lesion and precipitating thrombosis. Both of these models can be used to study the acute events that occur after plaque rupture: however, they do not represent the underlying pathobiology of spontaneous plaque rupture.

The apolipoprotein E-deficent mouse

The wild-type laboratory mouse exhibits a different distribution of cholesterol amongst its lipoproteins compared to man, and is highly-resistant to the spontaneous development of complex atherosclerotic lesions [13]. However, the C57BL/6 strain of mouse develops small fatty streak-like lesions when fed for prolonged periods on a high-fat/high-cholesterol diet [14].

In the early 1990s, mice were genetically engineered that were deficient for the apolipoprotein E (apoE) gene [15-17]. apoE serves as a ligand for the LDL receptor-mediated removal of chylomicrons and very low-density lipoproteins (VLDL). Consequently, apoE-deficient ($apoE^{-/-}$) mice spontaneously develop hypercholesterolaemia, even on a low-fat/low-cholesterol diet [15, 17]. They develop atherosclerotic lesions morphologically similar to human lesions, of all phases and at similar predilection sites, including the aortic sinus, the aortic arch, the brachiocephalic, subclavian, carotid, renal, iliac and proximal coronary arteries, and the thoracic and abdominal aorta [18-20]. In addition, a high-fat/high-cholesterol diet markedly aggravates and accelerates lesion development [18]. Interestingly, in man there exists a rare familial disease where individuals exhibit mutations of the apoE gene and also present with hypercholesterolaemia and premature atherosclerosis [21].

Since the advent of the $apoE^{-/-}$ mouse model of atherosclerosis, researchers have published in excess of 700 manuscripts, utilising techniques such as cross-breeding with other genetically altered mice, pharmacological intervention, and gene therapy. These studies have aided our understanding of the pathogenic processes and factors of atherosclerosis, including inflammation, apoptosis, and the involvement of MMPs. Furthermore, the $apoE^{-/-}$ mouse has enabled researchers to test whether molecules that were previously identified as guilty by association in human samples, actually play major roles in the atherogenic process.

Inflammatory cell recruitment

Since atherosclerosis is considered an inflammatory disease, numerous studies have attempted to identify modulators of the inflammatory process in atherosclerosis. The adhesion molecules and chemokines that induce the adherence, endothelial transmigration and proliferation of inflammatory cells during lesion development are some of the investigated factors. $ApoE^{-/-}$ mice lacking gene expression of intracellular adhesion molecule-1 (ICAM-1), E-selectin, or P-selectin, exhibit smaller aortic atherosclerotic lesions than wild-type controls [22]. In addition, monocyte chemoattractant protein (MCP)-1 and monocyte-colony stimulating factor (M-CSF) also promote atherosclerotic progression in the $apoE^{-/-}$ mouse [23-27]. Although macrophage-foam cell development and fatty streak formation are not completely eliminated, these studies confirm that the macrophage is a major player in murine atherosclerosis.

Inflammatory cell mediators

The role of lymphocyte involvement is more complex in atherosclerosis progression. Ablation of T and B cell populations in $apoE^{-/-}$ mice on a high-fat diet had no affect on lesion development [28, 29]. Conversely, on a low-fat/low-cholesterol diet, lesion size was decreased [28]. The possible atherogenic role of T cells has been further investigated by studying the CD40L-CD40 pathway in $apoE^{-/-}$ mice. In vitro stimulation of CD40L-CD40 signalling in cells derived from atherosclerotic lesions results in the expression of pro-atherogenic molecules such as adhesion molecules, chemokines, cytokines, MMPs and tissue factor [30]. Hence, $apoE^{-/-}$ mice that are also deficient for CD40L, or have been treated with an anti-CD40L antibody, have dramatically smaller lesions [31, 32]. In addition, treatment of established advanced lesions results in the development of plaques exhibiting features of a stable phenotype: they are VSMC and collagen-rich, lipid-poor, and have a decreased T cell/macrophage content [32]. Moreover, a concomitant increase in transforming growth factor (TGF)-β expression was observed in treated animals compared to controls. TGF-β has several potentially anti-atherogenic properties, including stimulation of collagen synthesis [33, 34], inhibition of

MMP expression and enhanced endogenous tissue inhibitor of MMPs (TIMPs) expression [35]. Supporting this, when apoE$^{-/-}$ mice are treated with a neutralising antibody that inhibits TGF-β_{1-3}, atherosclerotic lesion development is accelerated [36]. Additionally, in a study utilising a competitive inhibitor of TGF-β which inhibits its signalling, transition to a more unstable plaque phenotype was promoted [37]; there was a paucity of collagen deposition, whilst lipid content and inflammatory cell number were both increased. Furthermore, both old and new intraplaque haemorrhages were observed with associated fibrin deposition. The results from both the above studies suggest that TGF-b plays an important role in both inflammatory cell and ECM biology in murine atherosclerotic lesions.

Similarly, interventions in apoE$^{-/-}$ mice with the pro-inflammatory cytokines interferon (IFN)-γ [38], interleukin (IL)-1β [39, 40] and IL-18 [41], and the anti-inflammatory cytokine IL-10 [42], also modulate lesion development and the characteristics of plaque stability. These studies further illustrate the complex and multifactorial nature of inflammatory mechanisms during atherosclerotic lesion development.

Apoptosis

Apoptosis has been postulated to contribute to human atherosclerotic plaque growth and instability. The tumour-suppressor gene p53 has both anti-proliferative and pro-apoptotic actions, and co-localises with non-proliferating or apoptotic macrophages in human atherosclerotic lesions [43]. When p53-deficient mice were crossed with apoE$^{-/-}$ mice, large hypercellular aortic atherosclerotic lesions were observed [44]. Furthermore, the lesions exhibited increased proliferation, whilst no effect on apoptosis was detected. Similar results were found in the neointimal lesions of wire-injured p53-deficient mice [45]. However, contradictory results were seen in a recent study utilising placement of a restrictive silastic collar around the common carotid artery of apoE$^{-/-}$ mice, with local delivery of a recombinant adenovirus expressing human wild-type p53 [46]. Overexpression of p53, but not a control reporter gene, decreased cell proliferation and increased fibrous cap cell apoptosis and hence reduced cap thickness. These are features of an unstable plaque phenotype: interestingly, rupture-like events were also observed in 12.5% of these p53-induced lesions. The incidence of plaque rupture was further increased to 40% of lesions after intravenous injection of the vasopressor compound phenylephrine. Hence, p53 levels can regulate cell proliferation and apoptosis within murine atherosclerotic lesions, and therefore modulate plaque size and stability.

MMPs

Studies in human atherosclerotic plaques have suggested a decisive role for MMPs in plaque destabilisation. To elucidate their mechanistic role in the pathogenesis of lesion development, researchers have utilised gene overexpression of MMPs and TIMPs, gene deletion mice, and synthetic MMP inhibitors.

When macrophages created to overexpress MMP-1 were injected into apoE$^{-/-}$ mice, subsequent atherosclerotic lesion development was reduced [47]. This unexpected reduction in lesion size may have been due to increased collagen degradation or decreased collagenous matrix deposition. Additionally, anticipated destabilisation of the lesions was not observed, presumably due to retarded plaque development.

The ablation of MMP-3 in apoE$^{-/-}$ mice resulted in larger aortic atherosclerotic plaques. However, the lesions exhibited characteristics of a stable phenotype including increased collagen and decreased macrophage content [48].

Two groups have studied the effect of TIMP-1 gene deletion in apoE$^{-/-}$ mice, demonstrating local vessel wall MMP activity was increased [49, 50]. However, the results on plaque size were ambiguous, with no substantial beneficial effect on aortic atherosclerosis. TIMP-1 has also been overexpressed in apoE$^{-/-}$ mice by systemic delivery of an adenovirus expressing human TIMP-1, resulting in increased plasma levels of TIMP-1 [51]. Four weeks after adenoviral infection aortic plaque area was reduced in a small number of animals compared to uninfected mice. In addition collagen, elastin and VSMC content were enhanced whilst macrophage number was decreased, consistent with an increase in plaque stability. In contrast, oral administration of synthetic MMP inhibitors had no effect on lesion size or stability in hypercholesterolaemic mice [52].

These experiments highlight the potential complexities of manipulating a system where MMPs may have a dual role in plaque growth: supporting the growth of stable plaques through VSMC migration and matrix deposition, but promoting instability by ECM

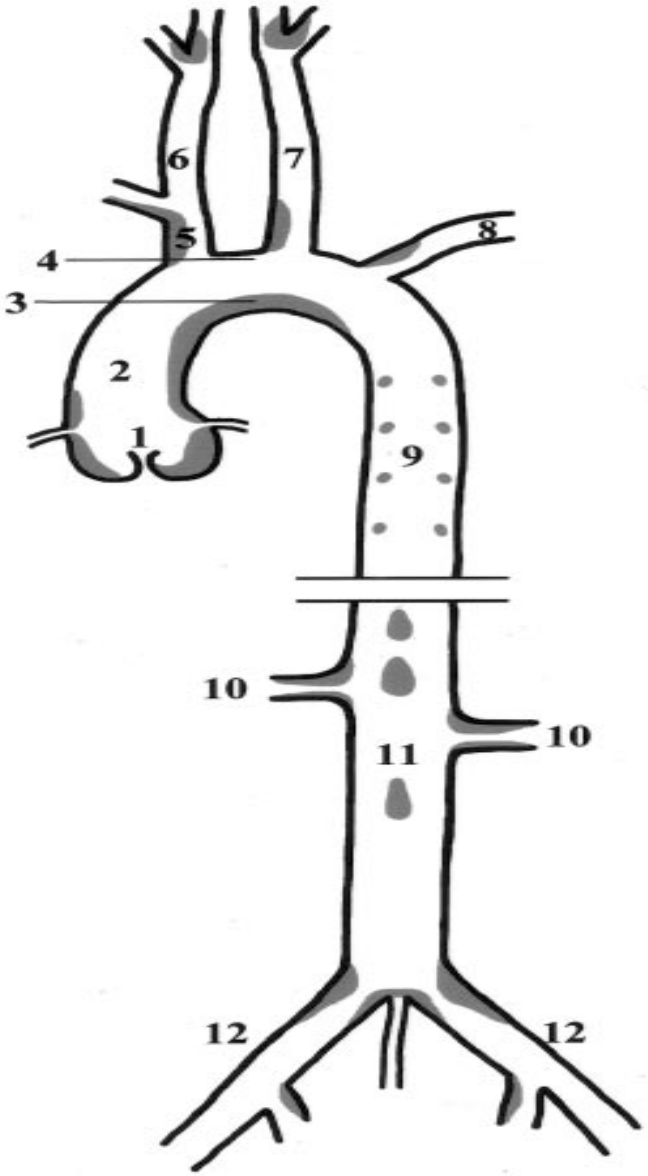

Figure 1. Distribution of atherosclerotic lesions in the apoE$^{-/-}$ mouse (gray shading), modified from (20)

1 = aortic sinus/root; 2 = ascending aorta; 3 = lesser curvature of aortic arch; 4 = greater curvature of aortic arch; 5 = brachiocephalic artery ; 6 = right common carotid artery; 7 = left common carotid artery; 8 = left subclavian artery; 9 = thoracic aorta; 10 = renal artery; 11 = abdominal aorta; 12 = iliac artery

destruction. The output from studies in which MMP biology is perturbed may thus be critically dependent on the initial conditions, such as the point in plaque development at which treatment is initiated, the nature of the atherogenic stimulus, the vessel studied, and the species.

Statins

The statins are a family of drugs that inhibit 3-hydroxyl-3-methylglutaryl coenzyme A (HMG-CoA) reductase, an enzyme pivotal to cholesterol biosynthesis. Numerous human trials have shown their potential for treating hypercholesterolaemia and ability to reduce and prevent coronary morbidity and mortality [53]. However, statins are thought to act through numerous pleiotropic effects, independent of cholesterol lowering. They have several anti-inflammatory effects, including reduced inflammatory cell adhesion and chemokine expression, increased nitric oxide (NO) synthase transcription, inhibition of SMC proliferation, and inhibition of vascular cell MMP expression (reviewed by [54-56]). When simvastatin was administered to cholesterol-fed apoE$^{-/-}$ mice, reduced leukocyte-endothelial cell interaction was observed, associated with enhanced NO release from vascular cells [57]. Furthermore, simvastatin reduced aortic cholesterol accumulation in apoE$^{-/-}$ mice without altering plasma cholesterol levels or lipoprotein profile [58]. In contrast, several studies have demonstrated that simvastatin (and other statins) increased plasma cholesterol levels and augmented plaque size in both the aorta and brachiocephalic artery [59-61]. The results from these studies suggest that the lipid-lowering effect of statins may depend on the presence of intact apoE. However, brachiocephalic lesions from simvastatin-treated apoE$^{-/-}$ mice demonstrate a reduced frequency of intraplaque haemorrhage and calcification, when compared to controls [61]. Both are markers of an unstable plaque phenotype, suggesting that simvastatin may stabilise established lesions. In addition, the expression levels of the anti-inflammatory molecules tissue factor and MCP-1 are attenuated in advanced brachiocephalic plaques after simvastatin treatment [62]. Therefore, although statins cause an elevation of plasma cholesterol levels in apoE$^{-/-}$ mice, they also exert both anti-inflammatory and anti-thrombotic effects, favouring transition to a stable plaque phenotype.

Other factors

The studies decribed above highlight molecules that are protective and others that accelerate atherogenesis. However, spontaneous plaque rupture, the most significant aspect of human atherosclerosis, is largely absent in the models used. A suitable and reproducible model of plaque rupture is required to

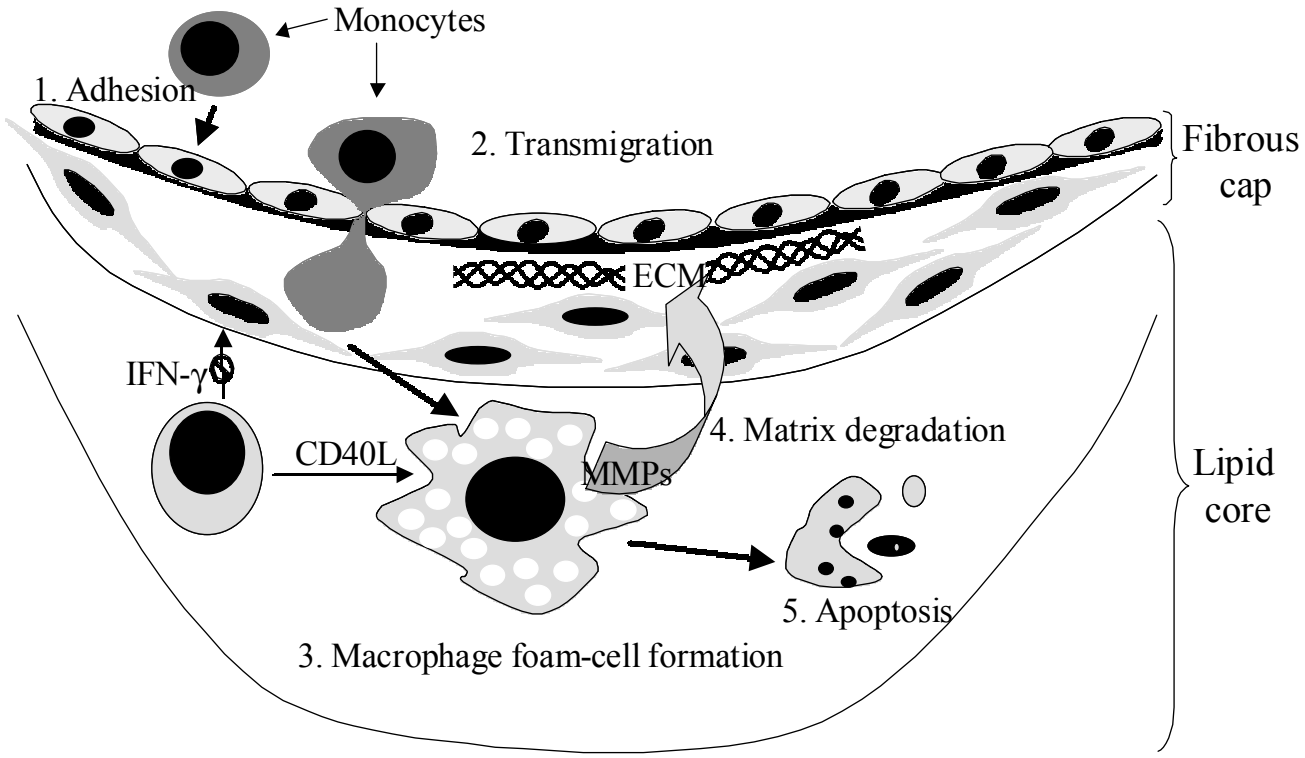

Figure 2. A schematic diagram of processes involved in atherosclerotic plaque development, progression and instability.

examine further the role of these targets in a setting of lesion destabilisation, and to enable the testing of potential therapeutic interventions.

Mouse models of plaque destabilisation

In 1998, the first evidence of atherosclerotic plaque-associated thrombus formation in the apoE$^{-/-}$ mouse was published [69]. Squeezing the atherosclerotic area of the aorta in vivo with forceps induced plaque disruption. This injury induced release of plaque contents, foam cells and foam cell contents promoting the formation of platelet-fibrin thrombi, demonstrating thrombogenesis after plaque disruption, albeit by unphysiological means. Another method of stimulating plaque rupture, as mentioned earlier, involved collar-induced carotid artery lesions in apoE$^{-/-}$ mice over-expressing p53 [46]. Although intraplaque haemorrhage was observed in a number of lesions, rupture was only seen after the animals were rendered hypertensive by treatment with phenylephrine. Several studies have also illustrated that hypercholesterolaemic mice suffer myocardial infarction, but omit any evidence of spontaneous plaque rupture [70-72].

A recent study utilised mice deficient for both apoE and the LDL receptor that develop extensive atherosclerosis throughout their vasculature [73]. When the mice were fed a high-cholesterol diet for up to 12 months, a small minority of animals showed plaque rupture with possible mural thrombi in the aorta. The same group also studied the coronary arteries from long-term (up to 20 months) chow-fed apoE$^{-/-}$ mice. They observed coronary atherosclerosis in 55% of animals, a small number of which exhibited blood-filled channels. This was taken to suggest recanalisation following previous plaque disruption and thrombosis. Interestingly, a minority of the animals also showed signs of plaque rupture or thrombus formation in the aortic sinus.

The majority of past studies in the apoE$^{-/-}$ mouse have focused on atherosclerotic lesions at the aortic sinus/root. Recently, attention has moved to the brachiocephalic artery, which is the first branch point

from the aorta and is approximately 200 μm in length (**Figure 1**). The lesions found at this site are advanced and complex, reproducible, and closely mimic those found at the human carotid bifurcation [20]. It was recently demonstrated that the brachiocephalic arteries from older chow-fed apoE$^{-/-}$ mice harbour atherosclerotic lesions exhibiting a high frequency (75%) of intraplaque haemorrhage [74]. The lesions are characterised by the presence of collagen-rich fibro-fatty nodules flanked by lateral xanthomatous-like new growths. Numerous lesions exhibited intramural bleeding, which was interpreted as a consequence of plaque rupture. Furthermore, layered lesions were seen in many of the animals, suggestive of multiple rupture events, as observed in human coronary atherosclerosis [75]. However, neither plaque rupture per se, nor lumenal thrombosis, was identified in any of the lesions.

Although these studies have illustrated some features of plaque destabilisation, numerous caveats remain. Convincing evidence for the formation of platelet-rich fibrin clots, an important component of human plaque rupture, remains elusive in the mouse models of atherosclerosis commonly used today.

A mouse model of atherosclerotic plaque rupture

We have recently demonstrated that a high proportion of apoE$^{-/-}$ mice on a mixed strain background (79% C57BL/6 : 21% 129) suffer spontaneous plaque rupture in their brachiocephalic arteries when fed a high-fat diet for up to 52 weeks [76]. When compared to other sites in the vasculature, rupture is much more frequently observed in the brachiocephalic artery. Furthermore, at these rupture sites, lumenal thrombi are observed that communicate with the lipid core of the plaque at a point of disruption in the VSMC-poor fibrous cap. This study demonstrates the existence of an anatomical site of predilection for spontaneous plaque rupture in the brachiocephalic artery of apoE$^{-/-}$ mice.

We further characterised the development and morphology of brachiocephalic artery lesions in a large cohort of mice [77]. Over 50% of animals had an acutely ruptured plaque in the brachiocephalic artery. In addition, ruptured plaques were larger and more occlusive, had a greater lipid core and exhibited a higher frequency of previous episodes of silent plaque rupture when compared to intact lesion. This second study confirmed that spontaneous plaque rupture is a frequent occurrence within the brachiocephalic arteries of mixed strain apoE$^{-/-}$ mice. Furthermore, it highlights the reproducibility of this model and its potential use for the study of treatments for atherosclerotic plaque stabilisation.

Recently, we have witnessed acute plaque ruptures in the brachiocephalic arteries of our mice after only eight weeks of high-fat feeding. This evidence adds further weight to its suitability for studies focusing on the mechanisms of atherosclerotic plaque rupture.

Concluding remarks

Atherosclerosis is a complex multifactorial disease that involves a myriad of processes and factors, including inflammation, apoptosis-driven expansion of the lipid/necrotic core, and proteolytic breakdown of the ECM. Escalation of these mechanisms results in the rupture of the atherosclerotic lesion and subsequent thrombotic occlusion of the vessel. The studies discussed in this review have utilised apoE$^{-/-}$ mice to highlight molecules involved in the above mechanisms, influencing atherosclerotic lesion initiation, growth and development. The recent discovery of a reproducible site of predilection for plaque rupture, the brachiocephalic artery, will now allow further dissection of plaque development and destabilisation, allowing the elaboration and testing of therapies aimed at limiting this disease.

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Jason Johnson is a PhD Student, jointly supervised by Drs Christopher Jackson and Sarah George at the University of Bristol

Bristol-Myers Squibb

The Bristol-Myers Squibb Cardiovascular Prize Fellowship Award 2004

The Selection Committee members are pleased to announce that **Dr Joseph De Bono** is the recipient of the 2004 award.

Dr De Bono will study the role of oxidative stress and nitric oxide synthase in the endothelial response to exercise. His research will be supervised by Professor Keith M Channon in the Department of Cardiovascular Medicine, University of Oxford, John Radcliffe Hospital, Oxford.

This annual award funds candidates of registrar level to undertake full time clinical or laboratory based research for two or three years, and includes the running costs of projects leading to an MD or PhD.

The Selection Committee members are Professor M J Brown (Cambridge) (Chair), Professor K Fox (Edinburgh), Professor M Frenneaux (Cardiff), Professor H Watkins (Oxford) and Professor P Weissberg (Cambridge).

Details about applications for the 2005 award will be posted later in the year; further inquiries may be addressed to Dr Giovanni Sperti, Medical Department, Bristol-Myers Squibb Pharmaceuticals Ltd, 141-149 Staines Road, Hounslow, Middlesex TW3 3JA.

Travel Reports for *The* Bulletin

The Bulletin regularly publishes travel reports written by members. These are up to 3 pages in length, may include photographs and can be on any conference, course or laboratory visit of interest to other members. If you are planning on travelling to a cardiovascular-related meeting and would like to write a report for the Bulletin, please contact the editors. A bursary of **£100** is available towards the cost of your visit, and this will be provided on receipt of the report. Bon voyage!

BSCR Autumn 2003 Meeting:

'OXIDATIVE STRESS: FROM MEASUREMENT TO MANAGEMENT'

The University of Edinburgh, 8-9th September

A report by Gillian Gray (University of Edinburgh)

Early September saw the first joint meeting between the BSCR and the Scottish Cardiovascular Forum (SCF), held at the John MacIntyre conference centre in the shadow of Arthur's Seat in Edinburgh. In a change to the usual style and to allow for addition of a short SCF programme the meeting started bright (yes it was Scotland) and early on Monday 8th September with a welcome from David Webb, Head of the Edinburgh Centre for Cardiovascular Science. 75 delegates attended from all over the UK, with a particularly large contingent from Wales, obviously a hot bed of oxidative stress research. They certainly contributed significantly to the meeting, and took the opportunity to sample the Edinburgh nightlife.

Rudolph Riemersma (Edinburgh) kicked off the science, delivering the SCF keynote lecture entitled 'Fish oils: cardioprotective or not?' He demonstrated that while they can be cardioprotective due to their content of n-3 fatty acids, this benefit is reduced due to the accumulation of mercury in fish higher in the food chain. These factors have confounded the outcome of the various intervention trials designed to investigate the ability of fish oils to reduce coronary heart disease (CHD).

The joint SCF/BSCR free communication session which followed covered a variety of oxidative stress related topics from phospholipid chlorohydrin toxicity (Corinne Spickett, Strathclyde), through pulmonary disease (Phil Milliken, Strathclyde and Saibel Biswas, Edinburgh) to EPR spin traps (Ian Megson, Edinburgh) and NO in heart failure (Dev Datta, Cardiff). The BSCR Young Investigator Prize for oral presentation was awarded to Jeffrey Khoo from the John Radcliffe Hospital, Oxford for his presentation on 'EPR quantification of nitric oxide release from mouse aortas'. The standard of lunch-time poster presentation was high and several were very highly commended, the BSCR prize was awarded to Katie Shaw (Edinburgh) for her presentation on 'Nitric oxide and atherosclerosis:impact of NO-related species on disease development'. After a sunny lunch on the terrace the main BSCR meeting got under way.

The British Cardiac Society Lecture 'Redox signalling in mending the broken heart' was delivered by Dipak Das (Connecticut, USA) who had travelled via South America and Paris to be with us. Of particular interest to the audience was a series of elegant experiments demonstrating the induction of thioredoxin-1 (Trx-1) in the heart following preconditioning, and its role in cardioprotection shown using pharmacological blockade and mice with cardiac specific overexpression of Trx-1.

Valerie O'Donnell (Cardiff) then gave an impressive presentation outlining the work of her lab in investigating enzymatic mechanisms of NO consumption in vascular dysfunction. Among several examples given were the consumption of NO by neutrophil NADPH oxidase to permit neutrophil adhesion to the endothelium and the reduction of the hypertensive effects of angiotensin II in mice null for 12/15 lipoxygenase, another key regulator of NO removal.

The vascular theme continued with the presentation from Ingrid Fleming (Frankfurt) on the generation of reactive oxygen species by Cyp 2C, a cytochrome P450 expressed in endothelial cells, including novel data showing restoration of endothelial function in patients with CHD using a Cyp 2C inhibitor,

sulfaphenazole. Suppression of oxidative stress was suggested as an additional mechanism of the HMG-Co A reductase drugs (statins) by Ralf Brandes (Frankfurt). Redox-sensitive signalling was elevated on drug withdrawal and this was prevented in mice deficient in NADPH oxidase, suggesting this enzyme as the source of oxidative stress and a target for statins actions.

In the second session the theme changed from sources of oxidant stress to means of detecting it. Sandy Hill (Dundee) started by discussing the advantages of measuring isoprostanes as plasma markers of lipid peroxidation, using GC-MS, while acknowledging that technical advances need to be made before this labour intensive technique can be employed as a routine screen. Electron paramagnetic resonance (EPR) is a technique that is becoming more widely applied as testified by the abstracts submitted to this meeting. Joerg Muller (Magnettech, Berlin) described the basis for detection of free radicals using various spin traps and brought along a benchtop EPR to demonstrate its use. Irfan Rahman (Edinburgh) highlighted the importance of the oxidant/antioxidant balance in determining risk in cardiovascular disease. He went on to give an excellent overview of mechanisms of measuring anti-oxidant capacity in potential target tissues and products of lipid peroxidation in biological fluids, recommending that no one method can give a comprehensive assessment of oxidative stress and that caution should be exerted when trying to extrapolate in vitro data to in vivo situations.

Presentations on Tuesday moved the theme from detection techniques to their application in studies investigating the roles of oxidant stress in cardiovascular disease. Women who develop pre-eclampsia demonstrate an exaggerated inflammatory response to pregnancy associated with overt oxidative stress. Lucilla Poston (GKT, London) explained some of the reasons for this response and went on to show very clear data from her own group demonstrating reduction of isoprostane concentration and disease incidence in women at risk after a period of anti-oxidant prophylaxis. As a result the group has recently embarked upon a multi-centre randomised placebo trial of vitamin C and E in 2400 women.

Diabetes is another disease with characteristic alterations in oxidant/antioxidant balance. Mary Cotter (Aberdeen) presented evidence for the influence of oxidant stress on nerve fibre function and endoneurial perfusion. Identification of the hydroxyl radical as being of particular relevance, and of nuclear factor kappa B as a redox sensitive target may have implications for the targeting of drugs for treatment of diabetic neuropathy.

Moving from the vasculature to the heart Phil Eaton (London) focused on thiol groups as targets for oxidative damage. His group have developed sensitive assays allowing them to identify a number of proteins that are susceptible to S-thiolation in ischemia and reperfusion injury, including actin, GAPDH, HSP27 and a number of signalling molecules. Cardioprotection by carbon monoxide (CO) was the topic for presentation from Roberto Motterlini (Northwick Park). Data presented suggesting that transition metal carbonyls could be used as carriers to deliver CO for therapeutic use provoked a healthy debate. The subject of NADPH oxidase (NOX) and hypertrophy was elaborated elegantly by Ajay Shah (London), who after describing the insights gained from NOX subunit, gp 91phox, knockout mice, went on to demonstrate the association of another isoenzyme, NOX4, with ventricular hypertrophy induced by aortic banding.

The meeting was rounded up by consideration of various means of managing oxidant stress. Justine Davies (Dundee) presented a pharmacological approach using the xanthine oxidase inhibitor, allopurinol, to improve endothelial function in patients with heart failure, leading to the prospect that the inhibitor might reduce cardiovascular events and possibly improve exercise capacity. Simon Maxwell (Edinburgh) discussed the potential of uric acid as an anti-oxidant in cardiovascular disease. An alternative gene transfer approach was proposed by Julia Brosnan (Glasgow). Succesful delivery of superoxide dismutase into carotid arteries of stroke prone SHR was shown to improve endothelial function.

The final session was brought to a very satisfactory close by Jane Armitage (Oxford CTSU) delivering the National Heart Research Fund lecture on the topic of 'Anti-oxidant trial and epidemiology'. After a review of the early data that lead to clinical trials, various of the larger trials which have failed to show benefit were presented, considering the reasons why and what might be learnt. The presentation finished on a positive note with recent trials showing benefits of a fruit and vegetable rich diet against cardiovascular disease. A lively debate on anti-oxidant vitamins ensued, and continued into the therapeutic red wine sampling

which followed. Power cuts not withstanding, the emails received since the meeting suggest that it was very well received. The delegates are to be congratulated on their enthusiastic participation in debate during the scientific sessions and on the excellent standard of presentations. Outside of the science a good time was had by all at the reception and dinner held in the National Trust for Scotland Headquarters in Charlotte Square. The organisers wish to thank the British Cardiac Society, the British Heart Foundation, the National Heart Research Fund, Organon Laboratories, Roche Vitamins, Scottish and Newcastle Breweries and Unilever Research for their generous sponsorship of the meeting.

Autumn 2003 BSCR meeting: abstracts

THE SIGNIFICANCE OF CYP 2C-DERIVED REACTIVE OXYGEN SPECIES IN THE REGULATION OF VASCULAR HOMEOSTASIS AND FUNCTION.

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Since the initial reports that renal cytochrome P450 (CYP) enzymes can metabolise arachidonic acid to substances which affect arterial tone, it has become increasingly clear that CYP enzymes expressed within the cardiovascular system play a crucial role in the modulation of vascular homeostasis. There is strong evidence suggesting that the activation of a CYP epoxygenase in endothelial cells is an essential step in nitric oxide and prostacyclin-independent vasodilatation of several vascular beds, particularly in the heart and kidney. CYP–derived epoxyeicosatrienoic acids (EETs) also modulate gap junctional communication and promote endothelial cell proliferation and angiogenesis. However, in addition to generating EETs, CYP 2C expressed in endothelial cells also generates physiologically relevant concentrations of reactive oxygen species, which have consequences on transcription factor activity and adhesion molecule expression. Moreover, in patients with manifest coronary artery disease, endothelium-dependent relaxation to acetylcholine is markedly attenuated and can be restored by infusion of the CYP 2C inhibitor sulfaphenazole.

CARDIOMYOCTYE HYPERTROPHY FOLLOWING INHIBITION OF NITRIC OXIDE SYNTHASE (NOS) BY L-NAME

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NOS inhibition, induced by chronic infusion of pharmacological inhibitors such as L-NAME into normotensive rats, has been found to induce cardiac hypertrophy in some, but not all cases; there are few reports however of assessment of cardiomyocyte parameters. The objective was to characterise the extent of cardiomyocyte hypertrophy in left (LV) and right ventricular (RV) cardiomyocytes, following administration of L-NAME (35-40mg /kg/ day) to 8 week old rats for 4 and 8 weeks. Systolic blood pressure (tail cuff sphygmomanometer) increased ($P<0.05$) after 2 weeks of treatment (190.7+2.4 mmHg, n=12 v 140.2+2.7mmHg, n=3). Compared to non-treated animals: (1) heart weight: body weight ratio was 33% greater ($P<0.05$) after 8 weeks (0.0048+0.0003 , n=6 v 0.0037 + 0.0001, n=8); (2) protein mass of cardiomyocytes increased ($P<0.05$) by 101% and 69% in LV and RV, above respective basal values (35.7 +2.9 μg/μg DNA n=7, 43.9 +1.9 mg/mg, n=6) after 8 weeks; (3) cardiomyocyte protein synthesis (incorporation of ^{14}C-phenylalanine) was greater ($P<0.05$) at 4 weeks (+ 27% LV, +49% RV) and this increase was augmented at 8 weeks (+237% LV, +260% RV); (4) there was a tendency towards induction of hypertrophic responsiveness to the b-adrenoceptor agonist, isoprenaline (ISO, 10^{-7} M), particularly in RV cells ($P<0.05$, ISO + protein synthesis by 31+5% n=7 at 4 weeks in L-NAME treated cells only). In conclusion, infusion of L-NAME (>35 mg/kg/day) elicits an early onset, rapidly progressing and severe hypertrophy not only of LV but also RV cardiomyocytes; this may reflect malignant hypertension of both the systemic and pulmonary circulation due to NOS inhibition within the vasculature as well as the heart itself; lower doses of L-NAME might be more suitable for studies in chronic stable disease.

REQUIREMENT FOR 12/15-LOX IN ANGIOTENSIN II-INDUCED HYPERTENSION IN VIVO

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Lipoxygenases (LOX) are non-heme iron-containing enzymes, catalysing oxidation of arachidonate or linoleate to bioactive lipid hydroperoxides and are implicated in atherosclerosis/diabetes. The role of LOX in hypertension is unclear. Studies demonstrate a possible role for 12/15-LOX in angiotensin II (AT2)-induced vasoconstriction *in vitro*. Hence, AT2-induced vascular dysfunction was investigated in 12/15-LOX knockout mice (12/15LOX$^{-/-}$) *in vivo*. Infusion of AT2 (1.1mg/kg s.c, via osmotic minipump) for 7 days significantly increased BP in WT mice (maximum 143±5.8 mmHg at day 4 vs. 107±2.0mmHg at baseline; $p<0.05$). AT2 infusion in 12/15LOX$^{-/-}$ mice also increased BP but was not significantly different from baseline (e.g. 122±5.3 mmHg at day 4 vs. 108±3.2 mmHg at baseline; $p>0.05$). No increases were observed in control mice. Moreover, aortic rings from 12/15LOX$^{-/-}$ mice demonstrated impaired vasoconstriction to both phenylephrine and AT2, and impaired endothelium-dependent vasodilation. AT2 infusion elevated cardiac hypertrophy, (heart:body weight ratio) in both 12/15-LOX$^{-/-}$ and WT mice, indicating that 12/15-LOX may not be involved in AT2-induced cardiac hypertrophy. These studies show 12-15-LOX is involved in AT2-dependent hypertension, but not cardiac hypertrophy and lend insight into the role of 12/15-LOX in vascular disease. Further studies on specific vascular beds will allow us to determine the mechanism/s involved.

PLACENTAL SUPEROXIDE IN PRE-ECLAMPSIA.

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We have investigated the NAD(P)H oxidase associated superoxide production in placental tissue as modulator of oxidative stress in normotensive pregnancy (n=19; gestational age $38^{+6}\pm0^{+1}$ wks) and pre-eclampsia (n=15; gestational age $34^{+3}\pm1^{+5}$ wks). Specificity for NAD(P)H oxidase was assessed using inhibitors (L-NAME, rotenone, allopurinol, DPI and TIRON). A considerable superoxide production inhibited by DPI and TIRON was measured in all placenta tissues. No differences for total superoxide production ($O_{2\ total}^{\bullet}$), maximal superoxide production ($O_{2\ max}^{\bullet}$), or the rate of superoxide production were found between groups. Women with more severe, early onset of disease had a higher $O_{2\ total}^{\bullet}$ as compared to those with a mild late onset disease. We conclude that human placenta contains a highly active NAD(P)H oxidase, which could be an important source of superoxide during pregnancy and pre-eclampsia. These pilot data justify further and more detailed investigation for the role of NAD(P)H oxidase and placental oxidative stress in complicated pregnancies.

PHOSPHOLIPID CHLOROHYDRINS AND THEIR TOXICITY TO HUMAN MYELOID CELLS

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Oxidised lipoproteins are increasingly thought to play a critical role in the development of atherosclerosis, and have been observed in atherosclerotic plaques in human. Additionally, atherosclerosis is now generally accepted as an inflammatory condition, in which immune cells play a significant role. HOCl, which is produced by the phagocytic enzyme myeloperoxidase, is released during stimulation of the phagocytic immune cells that are the predominant cell present in the early stages of inflammation. The powerful antimicrobial nature of HOCl has been well documented. However, the effects *in vivo* of phospholipid chlorohydrins, which are formed following HOCl attack on the unsaturated phospholipids of lipoproteins, have yet to be thoroughly investigated. The aim of this project was to study the cytotoxic effects of both phospholipid and fatty acid chlorohydrins, using both ATP-dependent and MTT cell viability assays, and to determine if any cytotoxicity resulted via apoptosis by assaying caspase-3 activity. It was found that chlorohydrins of both stearoyl-oleoyl phosphatidylcholine (SOPC) and stearoyl-linoleoyl phosphatidylcholine (SLPC), as well as chlorohydrins of their corresponding fatty acids, caused significant depletion of cellular ATP and a loss of viability in the range of 25-100μM. Preliminary data also shows that the lower concentrations of chlorohydrins resulted in apoptosis. Thus, such chlorohydrins could contribute to the progression of atherosclerotic lesions and the development of a necrotic core.

ALTERATIONS IN GLUTATHIONE METABOLISM MAY BE CENTRAL TO THE DEVELOPMENT AND PROGRESSION OF ATHEROSCLEROSIS

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Oxidative stress is recognised to be a key factor in cardiovascular diseases, including atherosclerosis. To date, the mechanism by which oxidative stress is triggered and maintained is unclear, although incapacitance of endogenous antioxidant system has been implicated. Glutathione (GSH) is a powerful intracellular redox recycling antioxidant with a secondary action mediated by the enzyme glutathione peroxidase (GPx). The importance of GSH in this capacity is highlighted by the complex system involved in its synthesis (γ- glutamylcysteine synthetase, γ-GCS), reduction (GSH reductase) and breakdown (γ-glutamyl transpeptidase). Here we have set out to test the hypothesis that synthesis of this crucial antioxidant is dysfunctional and is instrumental in causing oxidative stress associated with atheroma in the aortic arch of apolipoprotein-E (Apo E) deficient mice. Our results clearly show reduced GSH (control- 99 ± 28, ApoE- 24.1 ± 5.2 nmoles/mg protein, $p<0.05$), γ-GCS (control- 3.5 ± 0.1, ApoE-1.0 ± 0.2 nmoles/mg protein, $p<0.05$) and GPx activity (control- 2.5 ± 0.6, ApoE- 0.6 ± 0.2 nmoles/mg protein, $p<0.05$), and elevated specific lipid peroxidation products 4-hydrox-2-nonenal (4-HNE) (control-3.9 ± 0.6 & ApoE-68.1 ± 13.33 mmoles/mg protein , $p<0.001$) and malondialdehyde (MDA) (control- 4.1 ± 0.7 & ApoE- 84.1 ± 53.8 μmoles/mg protein, $p<0.001$) in 10, 20, 30 and 40- week-old male Apo-E deficient mice. While the low levels of GSH are likely to be attributable to decreased γ-GCS activity, reduced GPx activity may be implicated in elevated levels of 4-HNE and MDA. Further studies are underway to elucidate the molecular mechanisms underlying reduced GSH biosynthesis.

AUGMENTATION OF GLUCOSE METABOLISM WITH PERHEXILINE IMPROVES PHOSPHOCREATINE RECOVERY FOLLOWING EXERCISE IN PATIENTS WITH HEART FAILURE.

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Chronic Heart Failure (CHF) is associated with abnormal muscle energetics. We hypothesised that augmentation of glucose metabolism, which is more oxygen efficient than free fatty acid (FFA) metabolism, improves skeletal muscle energetics in CHF. The anti-anginal drug perhexiline inhibits mitochondrial FFA uptake and potentiates glucose metabolism. Patients with CHF (NYHA II-III, EF<40%) were randomised (double-blind) to perhexiline or placebo. 31-P Magnetic Resonance Spectroscopy of calf muscle was performed before and after dynamic exercise and phosphocreatine (PCr) spectra obtained. Treatment with perhexiline led to a reduction in mean PCr recovery halftime (PCr t½) of 28s (SEM ± 12.1s) following exercise. There was no significant change in PCr t½ in the placebo group (ΔPCr t½ = 0.43s SEM ± 5.83s). Using ANCOVA to correct for baseline covariance, the reduction of PCr t½ in the treated group was shown to be significant ($p=0.023$).

Conclusion: Increasing glucose metabolism with perhexiline improves skeletal muscle PCr recovery halftime following exercise in CHF. This is a load and muscle bulk independent measure of mitochondrial oxidative phosphorylation suggesting an improvement in skeletal muscle energetics.

EPR IN OXIDATIVE STRESS RESEARCH: WHICH SPIN TRAP?

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Electron paramagnetic resonance (EPR) is emerging as a powerful tool in free radical research. However, the technique is reliant on successful spin-trapping using agents that react with radical species (e.g. superoxide, hydroxyl, nitric oxide) to generate a 'stable' radical with a characteristic spin signal, the amplitude of which is related to its abundance. A wide variety of spin-traps are available and we set out to characterise some common examples (DMPO, Tempone-H, and CPH) with respect to their ease of handling, their relative selectivity for different radical species, and the resistance of their respective spin-signals to reduction by endogenous antioxidants. In our hands, Tempone-H and CPH generated much clearer EPR spectra than DMPO in response to superoxide, peroxynitrite and hydroxyl; CPH was particularly sensitive to superoxide. NO had little impact on Tempone-H or CPH. All the spin signals obtained were susceptible to inhibition by physiologically relevant concentrations of antioxidants, but CPH was the most resistant.

METABOLIC MANIPULATION WITH PERHEXILINE IMPROVES MAXIMAL OXYGEN CONSUMPTION AND SYMPTOMS IN PATIENTS WITH DILATED CARDIOMYOPATHY.

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We tested the hypothesis that augmentation of glucose metabolism, which is more oxygen efficient than free fatty acid (FFA) metabolism is beneficial in heart failure. Perhexiline augments glucose metabolism by inhibiting FFA uptake. 20 patients with DCM and CHF (NYHA II-III, EF<40%) were randomised (double-blind) to perhexiline or placebo. Exercise testing with respiratory gas analysis to measure Maximal Oxygen Consumption during peak exercise (VO2 max) and completion of the Minnesota Living with Heart Failure Questionnaire (MLWHF) were performed before and after 2 months of treatment.

	Perhexiline	**Pre Perhexiline**	**Post Placebo Pre**	**Placebo Post**
VO2 max **(ml/kg/min)**	16.6 (SEM ± 1.4)*	18.0 (SEM ± 1.7)*	15.8 (SEM ± 1.4)$^{+}$	15.3 (SEM ± 1.7)$^{+}$
MLWHF	44.2 (SEM ± 8.6)**	30.3 (SEM ± 8.6)**	46.8 (SEM ± 6.2)$^{++}$	40.4 (SEM ± 6.7)$^{++}$
	* P=0.005	** P=0.008	+ P=NS	++ P=NS

Patients recruited did not have angiographically significant coronary artery disease suggesting that the benefit observed is independent of the drug's anti-ischaemic properties.

EPR QUANTIFICATION OF NITRIC OXIDE (NO) PRODUCTION FROM MOUSE AORTAS

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With the increasing use of transgenic mice to study NO biology, progress in methods to reliably quantify NO in mouse tissues is crucial. We developed a technique based on electron paramagnetic resonance (EPR) spectroscopy, using colloid iron (II) diethyldithiocarbamate [$Fe(DETC)_2$] to trap NO produced in mouse aortas. Recordings were made at 77K with a tabletop spectrometer MS200 (Magnettech). A characteristic triplet $Fe(DETC)_2$-NO signal was seen from *C57BL/6* mice aortas incubated with $Fe(DETC)_2$, that increased 3.5-fold on stimulation with calcium ionophore A23187 [3.82 ± 0.13 vs. 1.10 ± 0.13 au (arbitrary units); $p = 0.0001$]. The signal increased linearly with incubation time ($r^2 = 0.94$), was inhibited by L-NAME and was abolished by endothelial removal. Stimulated aortas from *eNOS knockout* mice had a very low signal (0.51 ± 0.06 vs. 3.55 ± 0.21 au in littermate controls; $p = 0.0002$). In contrast, mice with transgenic *eNOS* overexpression had a 2-fold increase in signal (7.54 ± 0.76 vs. 3.74 ± 0.43 au in littermate controls; $p = 0.0014$). We conclude that EPR is a powerful technique for quantifying NO in vessels from genetically modified mouse models.

PREFERENTIAL METABOLISM OF NITRIC OXIDE TO NITROSYLHAEMOGLOBIN IN DIABETES MELLITUS- A POTENTIAL MECHANISM OF MICROVASCULAR DISEASE

BN Datta*, AB Milsom, MP Thomas, J Parton, J Peters, J Goodfellow, CJH Jones, PE James, Wales Heart Research Institute, University of Wales College of Medicine, Cardiff, UKIntroduction

Microvascular nitric oxide (NO) activity may depend upon reformation from its metabolic products. We hypothesised that Hb glycosylation leads to abnormal NO metabolism in diabetics independent of other vascular risk factors, and is associated with peripheral neuropathy (PN). Methods Venous blood was isolated from 34 subjects with type 1 diabetes, 26 with type 2 diabetes and 22 controls. HbFeIINO, nitrite and nitrate (NOx) were measured at baseline and following addition of increasing concentrations of NO ex-vivo. The metabolic capacity of Hb for NO was determined as the production of HbFeIINO relative to NOx, expressed as a line gradient. Results In diabetics, HbFeIINO production exceeded total NOx production compared to controls (line gradient for diabetes 0.39±0.04, controls 0.20±0.02 (mean±SEM, p=0.001). Relative HbFeIINO production was similar in both diabetic groups. It correlated with the degree of Hb glycosylation (p=0.001), and was independent of other vascular risk factors. HbFeIINO production was also associated with PN after correction for glycosylation (p=0.02). Conclusion Preferential HbFeIINO production is strongly related to Hb glycosylation, which will thus alter transport and delivery of NO to the microvasculature. The link between HbFeIINO and PN in this study is consistent with abnormal NO metabolism causing microvascular complications in diabetes.

MODULATION BY SUPEROXIDE OF NEURALLY-MEDIATED CEREBRAL VASODILATION IS ALTERED AFTER SUBARACHNOID HAEMORRHAGE

W. H. Miller[1], R. M Wadsworth[1]*, A. A. Preston[1], A. R. McPhaden[2], J. Willems[3], I. M. Macrae[4], [1]Dept of Physiology & Pharmacology, University of Strathclyde, Glasgow, [2]Dept of Pathology, Royal Infirmary, Glasgow, [3]Interdisciplinary Research Centre, Katholieke Universiteit Leuven, Kortrijk, Belgium, and [4]Wellcome Surgical Institute, University of Glasgow.

Superoxide is generated in SAH and superoxide quenching relieves cerebral vasospasm. Rabbits received two intracisternal blood injections two days apart (SAH); shams received CSF. Stimulation of intramural nerves of the basilar artery mounted in a myograph caused vasodilation (46+5 %) which was inhibited by the superoxide generator LY83583 10 μM in non-operated rabbits (20+10%) and in shams (21+6%) but not in SAH (47+8%). The nitric oxide synthase inhibitor L-NAME 300μM inhibited neurogenic vasodilation in non-operated rabbits (24+7%) and in shams (43+8%) but not in SAH (63+9%). In SAH there was increased immunoreactivity for extracellular (ec) SOD in the media, endothelium and "plump" cells in the adventitia; staining for nitrotyrosine was unaltered. The results suggest that superoxide restricts cerebral vasodilation but this action is lost following SAH, possibly due to upregulation of ecSOD. Changes in ecSOD and vasodilator neurotransmitters may be adaptive changes that protect the cerebral vasculature against excess superoxide following SAH. Supported by the British Heart Foundation (FS/97046).

NEURONAL NITRIC OXIDE SYNTHASE (NOS1) REGULATES BETA-ADRENERGIC CONTRACTION AND CA HANDELING IN ISOLATED MURINE VENTRICULAR MYOCYTESY.

H.Zhang, C.E.Sears, E.A.Ashley, Y.M.Kim, B.CasadeiDept. of Cardiovascular Medicine, University of Oxford, John Radcliffe Hospital, Oxford, OX3 9DU.

Cardiac neuronal nitric oxide synthase (NOS1) has been demonstrated to be functionally important in modulating basal cardiac contraction and Ca^{2+} transients, however, the role of NOS1 in the regulation of beta-adrenergic responses remains unclear. Here we compared cell shortening (field stimulation at 6Hz), Ca^{2+} transients (3Hz, Fura-2) and L-type Ca^{2+} current density (I_{Ca}, 3Hz) in left ventricular myocytes isolated from $NOS1^{-/-}$ mice and their littermate controls (C) in response to low (2 nM) and high (1 μM) concentrations of isoproterenol (ISO). All experiments were carried out at 35°C. Contraction was greater in $NOS1^{-/-}$ at both ISO concentrations (5.15±0.34%, vs. 4.13±0.44% in C at 2 nM and 9.81±0.84% vs. 8.79±0.93% at 1 μM, $p<0.05$ between $NOS1^{-/-}$ and C). Similarly, the increase in peak Ca^{2+} transient in response to ISO was significantly greater in $NOS1^{-/-}$ (F_{365}/F_{380}, 0.07±0.03 vs.0.008±0.01 in C at 2 nM and 0.3±0.11 vs. 0.23±0.05 at 1 μM, $p<0.001$ between $NOS1^{-/-}$ and C). I_{Ca} density increased in response to both ISO concentrations (-2.52±0.34 pA/pF in $NOS1^{-/-}$ vs. -2.10±0.24 pA/pF in C at 2 nM and -6.21±0.73 pA/pF vs.-4.80±0.50 pA/pF at 1 μM) though differences between $NOS1^{-/-}$ and C did not reach statistical significance. These findings show that the increase in contraction and intracellular Ca^{2+} transients is accentuated in LV myocytes from $NOS1^{-/-}$ mice both at submaximal and high ISO concentrations and they indicate that NOS1 (rather than NOS3) is the source of NO involved in the autocrine regulation of beta-adrenergic responses.

DIFFERENTIAL GENE EXPRESSION ACROSS THE LV WALL IN FAILING HUMAN HEARTS

Alison F. Wallace, Martin A. Denvir & Gillian A. Gray Centre for Cardiovascular Science, University of Edinburgh

In the human heart, wall stress is highest in the endocardium, decreasing towards the epicardium. The aim of the present study was to investigate of these differences in wall stress are reflected in gene expression in failing hearts. LV tissue was obtained from patients with heart failure of various aetiologies undergoing transplant surgery. Gene expression of three genes encoding 3 proteins were consistently increased in the endo relative to epicardium, brain natriuretic peptide and endothelin-1 (ET-1), peptides linked to hypertrophic remodelling, and also gelsolin, a protein involved in the degradation and disassembly of sarcomeric proteins. Interestingly, expression of the cytoskeletal protein, titin and particularly the N2B isoform linked to myocardial stiffness showed 2 distinct patterns of expression. In the first group it was more abundant in the endocardium and in the other in the epicardium. The changes were reflected in the expression pattern of the ET_A receptor. The gene encoding the ET_B receptor showed no particular transmural expression pattern.

These data indicate that elevated wall stress in the endocardium may be linked to the expression of genes involved in remodelling, while other factors may be responsible for regulation of genes involved in determination of myocardial stiffness, including the 'giant spring' protein titin.

NITRIC OXIDE AND ATHEROSCLEROSIS: IMPACT OF NO-RELATED SPECIES ON DISEASE DEVELOPMENT

C.A.Shaw*, A.G. Rossi, I.L. Megson. University of Edinburgh, Centre for Cardiovascular Science, Hugh Robson Building, George Square, Edinburgh

The disease atherosclerosis is characterised by the formation of lipid-rich plaques in the sub-endothelial space of conduit arteries. The inorganic free radical nitric oxide (NO) can be both anti- and pro-atherogenic depending on the concentration and NO species generated.

In this study we have used electron paramagnetic resonance (EPR) spin trapping to establish the synthetic compound GEA-3162 as a peroxynitrite ($ONOO^-$) generator. Using the same method we have found elevated levels of superoxide anions (O_2^-)/$ONOO^-$ in the aortae of atherosclerotic ($ApoE^{-/-}$) mice, indicating higher levels of oxidative stress in the vicinity of plaques.

Finally, using various classes of synthetic NO-donor drugs and the O_2^- scavenger superoxide dismutase (SOD), we have found the mechanism of NO-induced apoptosis in isolated human neutrophils to be superoxide dependent.

MODULATION OF HYPOXIC PULMONARY VASOCONSTRICTION BY SUPEROXIDE AND HYDROGEN PEROXIDE IN THE PERFUSED LUNG.

P.H. Milliken*, R.M. Wadsworth. Dept. of Physiology and Pharmacology, University of Strathclyde, 27 Taylor Street, Glasgow, G4 0NR.

Hypoxia upregulates enzymes that produce superoxide (O_2^-) in pulmonary smooth muscle and endothelial cells. Rat lungs were perfused and ventilated with 20% O_2 (normoxia), 10 minute challenges of hypoxia were performed by changing the gas flow to 5%CO_2, 95% N_2. Perfusate containing LY83583 (O_2^- generator) augmented hypoxic pulmonary vasoconstriction (HPV) from 2.7±0.5mmHg to 6.6±1.9 mmHg (n=11), this was reversed by the SOD mimetic MnTMPyP. LY83583 also reduced the vasodilation of the NO donor SNAP. These findings show O_2^- to modulate HPV, with the consumption of NO, which maintains a low pulmonary pressure, being a major factor. MnTMPyP alone significantly increased HPV (5.8±1.3mmHg, n=6), which was returned to control levels by catalase. This suggests a conversion of O_2^- to H_2O_2, which has been shown to have pulmonary vasoconstrictor actions. The SOD inhibitor DETCA attenuated HPV (1.0±0.3mmHg, n=7), which is attributable to a decreased level of H_2O_2. These results indicate the involvement of both O_2^- and H_2O_2 in the modulation of HPV. It is probable that high concentrations of O_2^-, as would be produced under these experimental conditions by LY83583, acts through NO sequestration, whereas lower concentrations of O_2^- act through conversion to H_2O_2.

P.H. Milliken is funded by the British Heart Foundation (FS/2001056).

CURCUMIN INDUCES GLUTATHIONE BIOSYNTHESIS AND PROTECTS AGAINST OXIDANT- AND TNF-α- MEDIATED IL-8 RELEASE IN ALVEOLAR EPITHELIAL CELLS.

Saibal Biswas [1], Ian Megson [1] and Irfan Rahman [2] [1] Cardiovascular Sciences, [2] Respiratory Medicine, University of Edinburgh Medical School, Edinburgh, UK.

Curcumin (diferuloylmethane) is a principal component of turmeric, a major spice of the orient. Curcumin is a polyphenol and has been shown to possess anti-inflammatory properties. Given its use in the Ayurvedic system of medicine against various respiratory diseases and infections, we hypothesise that, in addition to its anti-inflammatory property, curcumin may also have antioxidant properties. Lung alveolar epithelial cells (A549) were challenged with the oxidative and inflammatory agents H2O2 and TNF respectively for 4 and 24, at which time, IL8 release, total GSH levels, NF-kB transactivation by luciferase reporter assay and expressions of histone deacetylase (HDAC 2), and γ glutamyl cysteine ligase (GCL) were measured by RT-PCR. Curcumin was found to have a prominent protective effect against H2O2 mediated damage as compared to TNF. Curcumin inhibited NF-kB luciferase activity (39%) and partially inhibited IL8 expression (14.56%) due to H2O2 and not TNF, upregulated GCS expression (45%) in H2O2 treated cells as compared to control(22%) and did not appear to have any effect on HDAC 2 expression. Co- incubation of curcumin with trichostatin A did not alter HDAC-2 expression or NF-kB transactivation/IL-8 release,, suggesting that curcumin may not act via histone acetylation or the recruitment of HDAC proteins in the transcriptional initiation complex. Curcumin also increased the basal levels of GSH in A549 cells. The present study therefore suggests that curcumin might be a potent antioxidant with additional anti-inflammatory properties. Therefore curcumin has therapeutic potential as an agent with dual antioxidant and anti-inflammatory properties.

Supported by the British Heart Foundation and Pfizer Global Research and Development, UK.

BRITISH SOCIETY FOR CARDIOVASCULAR RESEARCH

SPRING 2004 MEETING

"Frontiers in Cardiovascular Signalling"

1st and 2nd April, 2004

Manchester University, Hulme Hall

Organising Committee: David Eisner and Cathy Holt, University of Manchester

Thursday 1st April

1.00 – 2.00 Registration and Lunch

2.00 – 2. 05 Welcome

Keynote lecture

2.05 – 2.50	**EDHF & Vascular Function**	Arthur Weston (*Manchester)*

2.50 – 5.00 **Session 1: Calcium**
Chair : Susan Wray

2.50	Localised Ca fluxes in cardiac myocytes	Clive Orchard (*Leeds*)
3.15	Ca signalling in vascular smooth muscle	Ted Burdyga (*Liverpool*)
3.40 – 4.10	Coffee/Tea	
4.10	Mitochondria and cardiac muscle	Michael Duchen (*London*)
4.35	**Mitochondria & Ca in vascular smooth muscle**	Tomoko Kamishima (*Liverpool*)

5.00 – 5.45 **British Cardiac Society Lecture**

Calcium signalling in the heart: basic mechanisms to heart failure
W J Lederer *(Baltimore, USA*)

5.45 – 7.00 **Poster Session** (wine reception)

7.30	Dinner	
9.00	Postprandial reflections on signalling	Austin Elliot *(Manchester*)

Friday 2nd April

9.00 – 10.40	**Session 2: Phosphorylation Signalling Pathways** Chair: Cathy Holt	
9.00	G proteins in cardiac myocytes	Angela Clerk (*London*)
9.25	Ras signalling in cardiac myocytes	Chris Proud (*Dundee*)
9.50	NFkB in vascular cells	Robin Plevin (*Glasgow]*
10.15	SAPKs in small arteries	Jaqui Ohanian (*Manchester*)
10.40 – 11.10	Coffee/Tea	
11.10 – 12.10	**Selected Abstracts** Chair: Stephen O'Neill	
12.10 – 12.55	**National Heart Research Fund Lecture** **Targeting signalling for restenosis** Robert Wilensky *(Philadelphia,USA)*	
12.55 – 2.00	Lunch	
2.00 – 4.30	**Session 3: Signalling out-of-control** **Chair: David Eisner**	
2.00	Ca signalling in heart failure	Andrew Trafford *(Manchester)*
2.25	MAPKs in ishaemia-reperfusion	Michael Marber *(London)*
2.50	**Smooth muscle signalling in atherosclerosis**	Quingbo Xu (*London*)
3.15 – 3.45	Coffee/Tea	
3.45 – 4.30	**Keynote Lecture** NO in heart failure	Jean Luc Ballingand *(Belgium)*
4.30 – 4.45	**Abstract Prizes and Meeting Close**	

Cardiovascular Related Meetings

XVIII World Congress International Society for Heart Research and the 52nd Annual Scientific Meeting of the Cardiac Society of Australia & New Zealand Cardiology Bench to Bedside: The Science and The Practice Brisbane Convention & Exhibition Center, Brisbane, Australia. August 7-11, 2004. Enquiries: ISHR 2004 Congress, PO Box 164, FORTITUDE VALLEY QLD 4006, AUSTRALIA. Phone.: +61 (0)7 3854 1611, Fax: +61 (0)7 3854 1507, E-mail: heart2004@ozaccom.com.au, Website: www.heart2004.com

Cellular Injury in Ischaemia (a satellite to the World Congress in Brisbane) will be held August 13-15, 2004 in the Kruger National Park, South Aftrica. Enquiries: Jacques van Rooyen: Dept of Physiological Science, University of Stellenbosch , Matieland, Stellenbosch 7602, South Africa, Fax: +27 21 808 3145 E-mail: jvrooy@sun.ac.za.

Endothelial Factors and Coronary Disease (a satellite to the World Congress in Brisbane) will be held August 13-15, 2004 in Hong Kong, China. Enquiries: Prof. Ricky Y.K. Man, Dept of Pharmacology, Faculty of Medicine, The University of Hong Kong, 2/F, Laboratory Block, 21 Sassoon Rd., Hong Kong China. Fax: 852 2817 0859, E-mail: ISHR-Satellite@hkuhk.hku.hk, Website: www.ISHR-satellite.hku.hk

Ageing Heart and Vessels: Current Understanding, New Research and the Challenge of Reducing the Health Care Impact of Age-Related Cardiovascular Disease will be held in Melbourne, Australia on Aug 3-5, 2004 as a Satellite Symposium of the World Congress of the International Society for Heart Research in Brisbane. Enquiries: Prof. Salvatore Pepe spepe@baker.edu.au ; Tel. +61385321310; Fax. +61385321314). Also, see www.baker.edu.au/ishr

Keystone Symposia: 'Molecular Biology of Cardiac Disease' and 'Cardiac Development and Congenital Heart Disease'. March 7-12, 2004, Keystone Resort, Colorado, USA. For further information: www.keystonesymposia.org; 221 Summit Place #272, Drawer 1630, Silverthorne, CO 86498; Tel: +1 970 262 1230; info@keystonesymposia.org.

XXVI Annual Meeting of ths ISHR - North American Section "Bench to Bedside and Back: Exploring new Paradigms - A Multifunctional Perspective of Cardiovascular Research in North America". May 2-5th, 2004. Westin Regina Resort, Cancun, Mexico. Enquiries: Dr Daniel Villarreal, SUNY Upstate Medical University Syracuse NY13210; Tel: (315) 464-9578; Fax: (315) 464-9571; E-mail: Villard@upstate.edu

XXVI Annual Meeting of ths ISHR - European Section will be held June 2-6, 2004 in Dresden, Germany. Enquiries: Dr. Ursula Ravens, Medical Faculty Carl Gustav Carus, Dresden University of Technology, Fetscherstrasse 74, 01307, Dresden, Germany. Phone: +49-351-458-6251, Fax: +49-351-458-6315, E-mail: ishr-dresden2004@mailbox.tu-dresden.de, Website: www.ishr-europe.org.

For up to date information on forthcoming meetings, workshops and symposia,

please remember to check the new BSCR Website:

http://www.bcs.com/affiliates/bscr.html

Cardiovascular Related Wellcome Trust Grants

September 2003 to November 2003

Principal Research Fellowship

Professor John J Mullins, aculty Of Medicine, University Of Edinburgh. Genetic And Cellular Mechanisms Determining The Functional Specialisation Of Kidney Juxtaglomerular Cells To Synthesise And Secrete Renin. 9 Months, £57,250.

Project Grant

Professor M J S Langman, Department Of Medicine, Queen Elizabeth Hospital, University Of Birmingham. Sudden Death In The Community. An Examination Of Drug Exposure As An Antecedent Factor. 36 Months, £18,944.

Dr David S Leake, Biochemistry And Physiology Research Group, School Of Animal And Microbial Sciences, University Of Reading. The Effects Of Hypoxia On The Response Of Cells To Oxidised Low Density Lipoprotein. 18 Months, £102,000.

Dr Sergey Kasparov, Department Of Physiology, School Of Medical Sciences, University Of Bristol. Central Phosphatidylinositide 3-Kinase (PI3K) Signalling Pathway In Genetically Pre-Programmed Hypertension. 36 Months, £169,894.

Research Career Development Fellowships In Basic Biomedical Science

Dr Ming Lei, Laboratory Of Physiology, University Of Oxford. The Ionic Basis Of Murine Sino-Atrial Node Pacemaking. (Supplement To Existing Award).28 Months, £64,969.

Dr Mark A Pook, Department Of Medical And Community Genetics, Kennedy Galton Centre, Level 8, Imperial College London, Harrow. Generation Of A Friedrich's Ataxia Transgenic Mouse Model By Using Transformation-Associated Recombination Cloning In Yeast. 36 Months, £6,659.

Professor K Ravi Acharya, Department Of Biology And Biochemistry, , University Of Bath. Structure-Function Studies On Human Angiotensin Converting Enzyme And The Design Of Novel Structure-Based Inhibitors. 36 Months, £145,160.

Dr Stefan P Hoppler, Department Of Biomedical Sciences, Institute Of Medical Sciences, University Of Aberdeen, Scotland. Wnt Signalling In Xenopus Organogenesis: Myocardium Specification. 36 Months, £245,401.

Professor Nicholas S Peters, Department Of Cardiology, St Mary's Hospital, Imperial College Of Science. Technology. And Medicine, London. Ageing And Atrial Fibrillation: The Role Of Changing Atrial Conduction In Mechanisms And Predictors. 24 Months, £187,140.

Dr Bridget M Lumb, Department Of Physiology, School Of Medical Sciences, University Of Bristol. Functional Anatomical Studies Of The Descending Control Of Nociceptive Inputs To Autonomic Control Centres In The Brain Stem And Its Behavioural Significance. 36 Months, £204,530.

Dr David L Buckley, Imaging Science And Biomed Engineering, , University Of Manchester. Kidney Perfusion And Glomerular Filtration In Renovascular Disease:Development Of A Comprehensive Single-Visit Renal Examination Using Mri. 24 Months, £115,367.

Research Career Development Fellowships In Basic Biomedical Science

Dr David Bates, Department Of Physiology, Veterinary School, University Of Bristol. Regulation Of Vascular Permeability By Shear Stress. 24 Months, £87,758.

Dr Jonathan M Gibbins, School Of Animal And Microbial Science, University Of Reading. Study Of The Peripheral Tachykinins Endokinin A And B In The Regulation Of Platelet Function. 36 Months, £195,879.

Travelling Research Fellowships

Dr Tetsuya Koyama, Department Of Anatomy And Developmental Biology, University College London. Long-Term Effects Of Shear Stress On Atp Release And The Expression And Function Of P2 Receptors In Vascular Endothelial Cells.24 Months, £106,724.

Dr M A Esteban, Renal Section, Division Of Medicine, Imperial College School Of Medicine, Hammersmith Hospital London. Characterization Of Enzyme-Substrate Interactions Underlying The Regulation Of Hypoxia-Inducible Factor By Oxygen Sensitive Hydroxylases. 24 Months, £91,638.

South African Senior Research Fellowships

Dr Edward D Sturrock, Department Of Medical Biochemistry, Medical School, University Of Cape Town Observatory South Africa. Angiotensin-Converting Enzyme: Crystallographic Studies, Structure-Guided Inhibitor Design, And Ectodomain Shedding.60 Months, £451,105.

Indian Senior Research Fellowships

Dr S V Ramanan, Au-Kbc Center For Internet And Telecom Technologies, Mit Campus, Chennai India. Intercellular Messangers That Couple Intercellular Cascades: From Permeability And Gating Of Gap Junction Channels To A Model Of Coordinated Tissue Response. 48 Months, £261,140.

Senior Central European Fellowships

Dr Maris Laan, Institute Of Molecular And Cellular Biology, University Of Tartu, Estonia. Haplotype Structure Of The Human Genome And Its Implications For Mapping And Understanding The Evolution Of Common Disease: Using Extensive Estonian Population Sample As A Model. 60 Months, £566,031.

Advanced Fellowships For Medical Graduates

Dr Bernard Khoo, Department Of Endocrinology, St Bartholomew's And Royal London School, Of Medicine And Dentistry, London. Mechanisms Of Splicing Inhibition In Apolipoprotein B Exon 26. 36 Months, £241,240.

Dr Roger S-Y Foo, Clinical Pharmacology Unit, Addenbrooke's Hospital, University Of Cambridge. The Role Of Cardiac Myocyte Apoptosis In The Transition From Compensated Hypertrophy To Dilated Cardiomyopathy. 36 Months, £273,323.

Dr S A Thom, Department Of Clinical Pharmacology, St Mary's Hospital Medical School, Imperial College School Of Medicine, London. Retinal Microvascular Abnormalities And Risk Of Cardiovascular Disease. 9 Months, £38,291.

Advanced Training Fellowship

Dr G D Batty, MRC Social And Public Health Science Unit, Glasgow. Cognition And Health: Analysis Of Data From A Series Of Population-Based Studies. 36 Months, £157,991.

Collaborative Research Initiative Grants

Dr Johanna C Moolman-Smook, Department Of Cardiovascular Medicine, John Radcliffe Hospital, University Of Oxford.Exploration Of N-Terminal Interactions Of Cardiac Myosin Binding Protein C (Mybpc) And Their Influence On Mybpc Quaternary Structure And The Regulation Of Cardiac Contractility. 36 Months, £108,131.

Professor A J Llanos, Department Of Physiology, University Of Cambridge. Effects Of Chronic Hypoxia On Fetal Cardiovascular And Endocrine Functions In The Sheep. 36 Months, £184,328.

Prize Studentships

Miss Catherine Panayiotou, Wolfson Institute For Biomedical Research, University College London. Role Of Soluble Guanylate Cyclase In Regulating An Inflammatory Response. 36 Months, £97,191.

Miss Cassandra Farthing, Department Of Cardiovascular Medicine, John Radcliffe Hospital, University Of Oxford. Cardiovascular Initiative - Genetic Mechanisms In Heart Development. 36 Months, £105,371.

Symposia

Dr Nicola J Brown, Department Of Surgical And Anaesthetic Scie, Royal Hallamshire Hospital, University Of Sheffield. 1 Months, £2,000.

Professor David Murphy Department Of Medicine, Bristol Royal Infirmary, University Of Bristol. Neurohumoral Control Of Cardiovascular Function - From Genes To Physiology. 3 Months, £3,000.

Professor Francesco P Cappuccio, Division Of General Practice And Primary Care, St George's Hospital Medical School London. British Microcirculation Society Meeting. Ethnicity And Cardiovascular Disease.1 Month, £1,000.

Cardiovascular Research Initiative

Mr Z Ali, Department Of Cardiovascular Medicine, John Radcliffe Hospital, University Of Oxford. Mouse Models Of Vein Graft Atherosclerosis. 36 Months, £168,481.

Dr David Adlam, Department Of Cardiovascular Medicine, John Radcliffe Hospital, University Of Oxford. In Vivo Regulation Of Vascular Haemodynamics By Endothelial Nitric Oxide Synthase. 36 Month, £180,893.

Articles for *The* Bulletin

Would you like to write a Review or Laboratory Profile for the BSCR Bulletin? These articles provide an excellent opportunity to let BSCR members know about your research activities and also provide an insight into your research field.

We are keen to hear from anyone in cardiovascular research who would be willing to write for *The* Bulletin.

If you are interested, please contact the Bulletin editors with your ideas: Helen (h.maddock@coventry.ac.uk) or Nicola (N.Smart@ich.ucl.ac.uk)

Submission Deadlines for *The* Bulletin:

Volume	*Date*	*Deadline*
17(2)	***April 2004***	*March 1st*
17 (3)	***July 2004***	*June 1st*
17 (4)	***October 2004***	*September 1st*
18(1)	***January 2005***	*December 1st*

BSCR Spring Meeting 2004

Frontiers In Cardiovascular Signalling

Dates: 1st and 2nd April, 2004

Venue: Hulme Hall, University of Manchester

Organisers: David Eisner and Cathy Holt

Overall Aims:
The aim of the meeting is to cover a wide variety of signalling pathways in both cardiac and vascular muscles. These include: calcium; phosphorylation signalling pathways and signalling out of control. As well as discussing current concepts and results, speakers will be encouraged to relate their findings to both normal physiology and disease.

Speakers include: Arthur Weston (*Manchester*), Clive Orchard (*Leeds),* Ted Burdyga (*Liverpool*), Michael Duchen (*London*), Tomoko Kamishima (*Liverpool*), W J Lederer (*Baltimore)*, Angela Clerk (*London)* Chris Proud (*Dundee),* Robin Pleven *(Glasgow*), Jacqui Ohanian (*Manchester*), Robert Wilensky (*Philadelphia*), Andrew Trafford (*Manchester*), Michael Marber (*London)*, Quingbo Xu (*London*), Jean Luc Ballingand (*Belgium*).

Travel & Accommodation: Hulme Hall, Oxford Place, Victoria Park, Manchester
The conference centre is located 1.5 miles for Manchester City Centre, 10 minutes walk from the main University Campus, with good public transport links. En-suite rooms and parking are available on site.

Communications: Part of this meeting will be devoted to oral presentation of selected abstracts, and posters. Prizes will be given for the best oral and best poster presentation given by young investigators.

Registration: Free to BSCR members, £40 for non-members.

Bursaries: The Society will consider awarding travel grants of up to £150 to bona fide PhD students.

Deadline for submission of abstracts, registration and application for student bursaries: 16th February, 2004.

The abstract pro-forma, meeting registration form, and forms for application for BSCR membership or student bursaries can be downloaded from: http://www.bcs.com/affiliates/bscr.html

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