

affect measured creatinine concentrations, notable offenders being delayed receipt of the sample, haemolysis and icterus. For this latter reason, many laboratories, including our own, do not report creatinine in samples with high bilirubin concentrations, although most report creatinines from samples which are only mildly icteric, are haemolysed or delayed. We evaluated these three common factors to assess their impact on measured serum creatinine levels, with subsequent effects on the eGFR. **Methods** We reviewed 50,000 consecutive serum creatinine requests received from Primary Care by the NHS Grampian Clinical Biochemistry laboratory. Of these, 37 were excluded as information was incomplete. The main analytical platform in this laboratory (Bayer Advia 24000) simultaneously assesses samples for the presence of haemolysis and/or icterus, rating the presence of each variable on a categorical scale of zero to + + + +. The mean creatinine levels for each of these categories were compared, to identify potential patterns suggestive of interferences. **Results** Mean creatinine levels for all samples was 100.0  $\mu\text{mol/l}$ . The results showed a negative effect of icterus with creatinine levels being on average lower in a progressive manner as the icterus increased (Figure 1, see [www.smj.org.uk](http://www.smj.org.uk)). A similar pattern was identified with regard to the presence of haemolysis in the samples (see Figure 2). Delayed receipt of samples appeared to result in a slight reduction in mean creatinine levels (Figure 3). **Conclusions** The three factors assessed all have graded influences on creatinine measurement. These effects may appear to be minor, but could have a significant effect on the classification of CKD by eGFR. For example, a 20 year old female with a creatinine of 100 would have a eGFR of 59  $\text{ml/min/1.73 m}^2$ , placing her in stage 3 CKD. This however, would not be detected if her sample was delayed more than 12 hours, or was only mildly haemolysed (1+ or more) or icteric (2+ or more), as the resulting eGFR would be greater than 60  $\text{ml/min/1.73 m}^2$ . Caution is therefore advised in the classification of CKD by eGFR in samples which are haemolysed, icteric, or simply delayed in transit.

## ABSTRACT OF SOCIETIES

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#### Dissociation of Phenotypic and Functional Endothelial Progenitor Cells in Patients Undergoing Percutaneous Coronary Intervention

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**Objectives** We sought to determine the effect of local vascular injury during percutaneous coronary intervention (PCI) on circulating endothelial progenitor cells (EPCs) in patients with stable coronary disease. **Background** EPCs are circulating mononuclear cells with the capacity to mature into endothelial cells, and to contribute to vascular repair. **Methods** EPCs were quantified and characterised by whole blood flow cytometry (CD34+KDR\* phenotype) complemented by real-time PCR, and the colony forming unit (CFU-EPC) functional assay, before and during the first 24 hours 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\*KDR\* cells or CFU-EPCs. Coronary intervention resulted in an increase in whole blood neutrophil count ( $\pm 1.31 \pm 0.35 \times 10^9/\text{L}$ ,  $P < 0.001$ ) 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 of CFU-EPCs increased 3-fold ( $0.6 \pm 0.2$  vs.  $2.3 \pm 0.9 \times 10^3$ ,  $P = 0.05$ ),

although circulating CD34\*KDR\* cells ( $0.019 \pm 0.003$  vs.  $0.021 \pm 0.003$  % of leucocytes,  $P = 0.75$ ) and leucocyte CD34 mRNA (relative quantity  $2.3 \pm 0.5$  vs.  $2.1 \pm 0.4$ ,  $P = 0.21$ ) did not. There was no correlation between CFU-EPCs and CD34\*KDR\* cells. **Conclusions** Acute 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\*KDR\* cells, suggesting these cells are not the primary source of circulating EPCs involved in the immediate response to vascular injury.

#### Mechanisms of Glucocorticoid-Mediated Inhibition of Angiogenic Changes in Endothelial Cells

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**Background** Manipulation of angiogenesis is an attractive therapeutic goal since aberrant blood vessel growth is central to many disease pathologies. The ability of glucocorticoids to inhibit angiogenesis is well established. This investigation addressed the hypothesis that glucocorticoid-mediated inhibition of angiogenesis can be modelled in endothelial cells, is the result of impaired VEGF action, and involves reduced endothelial cell migration. **Methods** Human umbilical vein endothelial cells (HUVECs) were cultured on Matrigel in conditions that stimulate spontaneous generation of tube-like structures (TLSs). The effects of glucocorticoids on TLS formation were assessed by incubation with cortisol (300-1200nM), cortisone (300-1200nM), vehicle or vascular endothelial growth factor (VEGF; 0.5-500ng/ml; positive control) for up to 24 hours. Effects on endothelial cell migration were assessed by culturing (24h) HUVECs on porous (8.0mm) inserts in the presence of VEGF (10ng/ml) +/- cortisol (600nM). HUVECs were then labelled with Calcein-AM and migration determined using fluorescence. **Results** Cortisol, but not cortisone, induced a concentration-dependent inhibition of TLS formation ( $44 \pm 7\%$ ,  $p < 0.01$ ) whereas VEGF stimulated an increase in TLS number ( $218 \pm 6\%$ ). HUVEC migration was enhanced ( $p < 0.01$ ) by exposure to VEGF (262% migration compared with untreated control cells). Cortisol had no effect on either basal (103%) or VEGF-stimulated (258%) migration. **Conclusions** These data show that TLS formation in endothelial cells provides a model for investigating the mechanisms underlying glucocorticoid-mediated inhibition of angiogenesis. The inability of glucocorticoids to reduce migration of these cells suggests their impact on angiogenesis may be due to changes in endothelial cell proliferation or on the formation of cell-cell connections.

#### Endothelial Dysfunction in Pre-eclampsia Prior to Clinical Symptoms

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**Background** Pre-eclampsia (PET) or high blood pressure in pregnancy results in approximately 150,000 maternal deaths worldwide and continues to be a leading cause of maternal and newborn illness or death in the UK. Endothelial dysfunction is a feature of PET and it is probably an early event in the development of PET. The aim of our study was to investigate if there was any evidence of endothelial dysfunction or white blood cell (WBC) activation prior to the onset of clinical signs in women destined to develop PET. **Methods & Subjects** 100 women with singleton pregnancies attending Ninewells Hospital for a scan were invited to take part in the study. At 22 weeks gestation a venous blood sample was collected from the mother along with the normal clinical data. Serum and plasma samples were stored at -70°C for measurement of vascular endothelial growth factor receptor-1 (VEGFR-1), neopterin and sE-selectin by ELISA. Sequential samples were taken from the same women at 26 and 34 weeks gestation and at 6 weeks postnatal. Following delivery pregnancy outcome was classified as PET, intrauterine growth retardation (IUGR) without PET or uncomplicated pregnancies. **Results** 83 women completed the study. 54 had a normal pregnancy; 14 had IUGR and 15 developed PET. sVEGFR-1 and neopterin levels were significantly higher at 26 weeks gestation in the PET group compared to controls, p values of 0.01 and 0.045 respectively. sE-selectin levels were significantly higher at 22, 26 and 34 weeks in the PET group compared with the controls ( $p = 0.02, 0.01, 0.024$  respectively). **Conclusion** In the past conflicting findings from various studies have been reported in women with PET. We have shown for the first time in a longitudinal prospective study that levels of the potent VEGF inhibitor sVEGFR-1, plus WBC and immune activation (reflected by increased neopterin levels) and endothelial

activation (as measured by sE-selectin) are all significantly higher before the development of clinical signs of PET. This could allow closer monitoring of pregnant women and perhaps the initiation of prophylactic treatment before the disease develops.

### Inflammation and Arterial Stiffness in Patients with Chronic Fatigue Syndrome

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**Background** Chronic fatigue syndrome (CFS) is characterised by lipid peroxidation with elevated levels of F<sub>2</sub>-isoprostanes that correlate with post-exertional myalgia. We have previously shown that many patients are in a pro-oxidant state consistent with significant cardiovascular risk. C-reactive protein (CRP) is a biochemical marker of chronic inflammation, which has been shown to be an independent predictor of cardiovascular risk. Markers of inflammation have been demonstrated in some CFS patients but little is known about the relationship between chronic inflammation and prognostic indicators of cardiovascular risk in CFS. Given the association between inflammation and increased arterial stiffness in other patient populations, and the recent evidence that increased arterial stiffness is an independent predictor of adverse cardiovascular outcome, we sought to investigate the relationship between CRP levels and arterial stiffness in well characterised CFS patients. **Methods** Forty one CFS subjects (age: 19-63 years) satisfied 1994 CDC criteria for CFS and 35 healthy volunteers served as controls. CRP (high sensitivity ELISA), 8-iso-prostaglandin F<sub>2a</sub> isoprostanes, total and high density lipoprotein (HDL) cholesterol, and oxidised low density lipoprotein (oxLDL) were assayed from plasma stored at -70°C. Arterial stiffness was measured by the SphygmoCor pulse waveform analysis system. Results: CFS patients had significantly increased levels of CRP ( $P < 0.01$ ) and 8-iso-prostaglandin F<sub>2a</sub> ( $P < 0.005$ ), compared with control subjects. Pulse wave analysis revealed significantly greater augmented pressure (AP) and augmentation index (AIx) in CFS patients than in control subjects ( $P = 0.045$  and  $P = 0.036$ , respectively) with AIx 61% higher in the CFS patients. In CFS patients, AIx showed univariate correlations with systolic blood pressure, CRP, isoprostanes and oxLDL, however, in a multiple regression model the significant correlation between AIx and systolic blood pressure, isoprostanes and ox LDL was removed and the only significant determinant of AIx was CRP ( $\beta = 0.449$ ,  $P = 0.007$ ). **Conclusion** CFS patients have significantly increased levels of plasma hs-CRP, and F<sub>2a</sub> isoprostanes that correlate positively with arterial stiffness. CRP was a predictor of arterial stiffness conferring a significantly increased risk of a future cardiovascular event for CFS patients.

### Vitamin D Improves Endothelial Function in Patients with Type 2 Diabetes in Scotland

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**Objectives** Vitamin D insufficiency is common in type 2 diabetes and is associated with an increased risk of cardiovascular events. We aimed to determine whether a single large dose of oral vitamin D could improve markers of vascular health, such as endothelial function, in patients with type 2 diabetes and vitamin D insufficiency (vitamin D  $< 50$ nmol/L). **Methods** This was a double blind, parallel group, randomised controlled trial. Patients with type 2 diabetes were recruited from Tayside Diabetes services between November 2005 and March 2006. Endothelial function measured by flow mediated vasodilation (FMD) of the brachial artery, office and 24hr blood pressure, and fasting bloods (calcium, phosphate, vitamin D, HbA1c, glucose, insulin, PTH) were assessed at baseline and 8 weeks after ingestion of 100,000U ergocalciferol or placebo. **Results** 43/87 (49%) of subjects screened had vitamin D  $< 50$ nmol/L and were included in the study. Mean age was 64.2 years, mean BMI 31.8 Kg/m<sup>2</sup> and mean baseline vitamin D 37.1nmol/L. Patients receiving vitamin D increased their serum 25-hydroxyvitamin D by 22.9nmol/L vs. 7.6nmol/L for the placebo group ( $p < 0.001$ ). Patients in the vitamin D group showed significant improvement in FMD compared to the placebo group (2.35% vs. 0.06%,  $p = 0.048$ ). Vitamin D

administration significantly decreased office systolic blood pressure (-7.3mmHg vs. +6.6mmHg,  $p = 0.001$ ), but did not significantly change 24 hour blood pressure. No significant difference was seen in insulin sensitivity, HbA1c or PTH between groups. **Conclusion** Supplementation with a single dose of oral vitamin D leads to significant improvements in endothelial function in patients with type 2 diabetes.

### Endothelial cell-specific ablation of the endothelin-B receptor does not alter expression or functional response of the endothelin-A receptor

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**Background** Endothelin-1 (ET-1) is a potent vasoconstricting peptide, produced by endothelial cells (ECs), which acts through ETA and ETB receptor subtypes and plays an important role in several cardiovascular diseases. Vasoconstriction and other detrimental effects of ET-1 are predominantly mediated by the ETA receptor whilst ETB receptors may have a protective role: promoting vasodilatation, natriuresis and clearance of ET-1. Transgenic over-expression of ET-1 has produced conflicting data on ETA expression, whilst down-regulation of this receptor has been consistently shown in "rescued" ETB knockout animals. We have previously generated a mouse featuring EC-specific knockout of the ETB receptor which is normotensive and demonstrates elevated plasma ET-1, secondary to impaired ET-1 clearance. We aimed to investigate whether the elevated plasma ET-1 in this model was associated with altered ETA receptor function or expression. **Methods** EC-ETB knockout mice were generated by a Cre-loxP approach. Isometric responses of femoral artery rings to ET-1 and the pressor effect of big-ET-1 in anaesthetised mice were recorded as measures of ETA receptor function. ETA gene expression was assessed by RT-PCR in kidney homogenates, and ETA receptor ligand binding quantified using autoradiography. **Results** Contraction of isolated femoral arteries to ET-1 was not altered in EC-ETB knockouts compared to littermate controls. The selective ETA antagonist, BQ123 reduced sensitivity of vessels to ET-1 and the magnitude of this effect did not differ between genotypes. Administration of bigET-1 induced a dose-dependent increase in blood pressure that did not differ between EC-ETB knockout animals and wild-type controls. Renal ETA receptor mRNA levels were unchanged in EC-ETB knockout compared to littermate mice, and this effect was maintained with ageing. Autoradiography demonstrated that ETA ligand binding was not altered in the kidney or lung of EC-ETB knockout animals compared to wild-type controls. **Conclusions** These studies demonstrate no alteration of ETA expression or response to ET-1 following EC-specific ETB knockout. This contrasts with "rescued" ETB knockout models, and suggests that over-exposure to ET-1 alone is insufficient to induce ETA down-regulation. Haemodynamic changes may play a further role in that ETA down-regulation is only present in models of elevated plasma ET-1 accompanied by overt or salt-sensitive hypertension. These data may have clinical relevance as elevated plasma ET-1 is described in many cardiovascular diseases and changes in expression of endothelin receptors may modulate the pathogenic effects of this peptide.

### Diesel Exhaust Inhalation Increases Thrombus Formation In Man

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**Background** Exposure to traffic-derived particulate air pollution may be a trigger for acute myocardial infarction although the mechanism is unclear. Diesel engines are considered to be the principal source of particulate air pollution in urban areas. In an animal model, tracheal instillation of diesel exhaust particles enhances arterial and venous thrombus formation in vivo. We investigated the effect of dilute diesel exhaust on thrombus formation in man using an ex vivo model of arterial thrombosis. **Methods** In a double-blind randomised crossover study, 7 healthy men were exposed to dilute diesel exhaust (1x10<sup>6</sup> particles/cm<sup>3</sup>) or filtered air ( $< 100$  particles/cm<sup>3</sup>) for 2 hours during intermittent exercise. Thrombus formation was measured at 6 hours using the Badimon ex vivo perfusion chamber at low (212 s<sup>-1</sup>) and high (1,690 s<sup>-1</sup>) shear rates with porcine aortic tunica media as the thrombogenic substrate. Aortic specimens were fixed and stained with combined Masson's trichrome-elastin stain. Total thrombus area was measured histologically using computerised

planimetry. All data are expressed as mean  $\pm$  SEM. **Results** Compared to air, exposure to diesel exhaust increased thrombus area under both high (27%  $\pm$  9%; n=7; P=0.02) and low (20%  $\pm$  9%; n=7; P=0.08) shear flow conditions.

**Conclusions:** Inhalation of diesel exhaust increases ex vivo thrombus formation in man. These findings provide a potential mechanism that links exposure to traffic-derived air pollution with acute atherothrombotic events including acute myocardial infarction. Figures to be found at [www.smj.org.uk](http://www.smj.org.uk)

### Haplotypic Variation Of The *Gli-1* Locus Determines Susceptibility And Phenotype In Ulcerative Colitis

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**Introduction** There is compelling evidence for a genetic susceptibility locus determining ulcerative colitis (UC) susceptibility/phenotype within 12q13 (IBD2; OMIM %601458), outwith the NOD2/CARD15 gene in the IBD1 region that is implicated in Crohn's disease (CD) pathogenesis. However, the causative IBD2 gene has yet to be identified. The hedgehog (HH) signalling pathway plays vital roles in gastrointestinal tract development, homeostasis and disease, Paneth cell differentiation, T cell immunology, and inflammation. The major HH pathway effector, *Gli-1*, lies within the IBD2 linkage region, leading us to hypothesis that *Gli-1* might play a role in disease causation. **Aims** To describe the expression patterns of key Hh signalling components in the healthy and inflamed colon, and analyse the contribution of inherited variants in the *Gli-1* gene to IBD susceptibility. **Methods** Expression of key Hh signalling components (SHH, IHH, PTCH, HHIP and *Gli-1*) were analysed by immunohistochemistry, microarray and real-time PCR in a large panel of formalin-fixed and fresh frozen colonic tissues from CD and UC patients, and healthy controls (HC). Initial mutation screening of the *Gli-1* gene was performed in 32 IBD cases. A total of 11 SNPs were typed, including 4 tagging SNPs (*rs3817474* [1SNP1], *rs2228225* [1SNP2], *rs2228224* [1SNP3], & *rs2228226* [1SNP4]) which defined *Gli-1* variation. Three common and five rare haplotypes were present in the Scottish HC population (n=1374). SNPs were assayed by Taqman in a Scottish IBD population [335 cases of CD & 474 of UC]. Taqman failures were assayed by direct sequencing. Haplotype & variant analysis was performed using chi2 analysis. Log-likelihood testing assessed the overall contribution of *Gli-1* variation to disease susceptibility. **Results** In detailed expression studies, we show that Indian hedgehog (IHH) - Patched-1 (PTCH) - *Gli-1* signalling is down-regulated in UC, supporting a causal role for hedgehog dysregulation in disease pathogenesis. It is of note that Sonic hedgehog expression increases with inflammation, consistent with recent reports that it is downstream of NF-KB. The gene-wide haplotype tagging strategy was employed to determine whether *Gli-1* is the IBD2 gene. We demonstrate a strong association with susceptibility to UC (OR=35.8; p<0.0001), particularly with severe disease (p=3.55x10<sup>-5</sup>), extensive disease (p=0.014), and requirement for colectomy (p=1.45x10<sup>-6</sup>). **Conclusion** These findings implicate common genetic variation in a key component of the HH signalling pathway in the pathogenesis of IBD.

### Characterisation of a Novel Regulator of Integrin Affinity

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The link between the surface of a cell and the extra-cellular matrix is vital in most tissues, and is mediated by the integrin family of cell surface receptors. Changes in integrin expression and function are implicated in several aggressive forms of cancer. Alterations to the way tumour cells adhere to their surroundings can promote metastasis and tumour invasion, and is important in how they respond to chemotherapy. Integrins also function to relay signals into and out of the cell to promote growth and differentiation. Using an expression library based screen we have identified a new integrin activator, the large multi-transmembrane domain protein "Nessie", which rescues integrin suppression induced by the small GTPase H-Ras. Our assay shows that expression of Nessie activates integrin affinity, despite localising to the Endoplasmic Reticulum (ER). We propose that Nessie may activate integrin affinity via another GTPase signaling molecule R-Ras, as a dominant negative form of R-Ras abrogates Nessie-induced integrin activation. Furthermore, Nessie expression causes reallocation of R-Ras to the ER. Nessie corresponds to the predicted gene KIAA0233 located on chromosome 16q23, a region that is associated with breast, gastric and lung cancer. Additionally, microarray data shows Nessie expression is down-regulated in many tumour lines. Our studies show that Nessie expression is markedly reduced in Small Cell Lung Cancer cell lines. Furthermore, knockdown of Nessie by siRNA causes drastic cell adhesion defects and promotes colony

formation. Additionally, Nessie siRNA produces defects in chromosome morphology, but cells still progress through the cell cycle and have reduced levels of apoptosis. These results provide strong evidence that loss of Nessie expression is linked to a metastatic tumour phenotype, and this novel protein may be a potential therapeutic target.

### Regulation of Alternative Macrophage Activation by Galectin-3 Controls Renal Fibrosis Following Ureteric Obstruction

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Macrophages display broad phenotypic heterogeneity depending on their microenvironment. Different functional subsets of macrophages may have opposing activities; pro-inflammatory versus anti-inflammatory, tissue destructive versus tissue reparative activities, which can be potentially modulated by cytokines. The pro-inflammatory activities of classically activated (M1) macrophages are enhanced in the presence of microbial agents and Th1 cytokines such as (IFN-g) and are essential for bacterial killing. In contrast, alternatively activated (M2) macrophages, which are enhanced by Th2 cytokines such as IL-4 and IL-13 are anti-inflammatory and are involved in debris scavenging, angiogenesis and tissue remodelling. Macrophages stimulated with IL-4 or IL-13 up regulate a number of phenotypic markers (YM-1, FIZZ1 and mannose receptor). The Th1 and Th2 cytokines also induce opposing macrophage phenotypes through up-regulation of NOS2 or arginase1 respectively. Competition between NOS2 and arginase 1 for the common substrate L-arginine results in the production of L-ornithine, a necessary metabolite for the production of proline (a critical amino acid for the synthesis of collagen), which links arginase activity to fibrosis. Galectin-3 is a b-galactoside-binding lectin which is highly expressed on macrophages and has been implicated, in a broad range of pathophysiological processes including acute and chronic inflammation and fibrosis. Our previous work has shown Galectin-3 is upregulated in human fibrotic disease, and disruption of the Galectin-3 gene reduces fibrosis in mouse models of liver, lung and renal fibrosis. The present work shows that macrophages from Galectin-3<sup>-/-</sup> mice show a specific deficit in IL-4- and IL-13-induced alternative macrophage activation and siRNA-mediated knockdown of Galectin-3 or its receptor CD98 prevents IL-4-mediated alternative macrophage activation. In a model of renal fibrosis induced by unilateral ureteric obstruction (UUO), Galectin-3<sup>-/-</sup> mice showed less fibrosis and reduced expression of the alternative activation markers YM-1, FIZZ1 and mannose receptor. Specific macrophage depletion in CD11b-DTR mice demonstrated that the macrophage is essential for fibrosis following UUO and adoptive transfer of wild type BMDMs into Galectin-3<sup>-/-</sup> mice was sufficient to restore the fibrotic phenotype. Galectin-3<sup>-/-</sup> may therefore control the development of fibrosis by promoting alternative macrophage activation.

### Liver Proteomics for Therapeutic Drug Discovery: Inhibition of the Cyclophilin Receptor CD147 Attenuates Sepsis-Induced Acute Renal Failure

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**Background** Sepsis-induced multi-organ failure continues to have a high mortality. The liver is an organ central to the disease pathogenesis. The objective of this study was to identify the liver proteins that change in abundance with sepsis and, therefore, identify new drug targets. **Methods** We used a mouse model of sepsis based on cecal ligation and puncture (CLP) but with fluid and antibiotic resuscitation. Liver proteins that changed in abundance were identified by difference in-gel electrophoresis (DIGE). We compared liver proteins from 6 hr post-CLP to sham-operated mice ('early proteins') and 24 hr post-CLP with 6 hr post-CLP ('late proteins'). Proteins that changed in abundance were identified by tandem mass spectrometry. We then inhibited the receptor for one protein and determined the effect on sepsis-induced organ dysfunction. **Results** The liver proteins that changed in abundance after sepsis had a range of functions such as acute phase proteins, coagulation, ER stress, oxidative stress, apoptosis, mitochondrial proteins and nitric oxide metabolism. We found that cyclophilin increased in abundance after CLP. When the receptor for this protein, CD147, was inhibited sepsis-induced renal dysfunction was reduced (serum creatinine 24 hr post-CLP: sham antibody 0.59  $\pm$  0.07mg/dl; anti-CD147 antibody 0.39  $\pm$  0.05 mg/dl n=20 p=0.04). There was also a significant reduction in serum cytokine production when CD147 was inhibited (serum TNF-a 24 hr post-CLP: sham antibody 225  $\pm$  40 pg/ml; anti-CD147 antibody 105  $\pm$  25 pg/ml n=8 p=0.03. Serum IL-6 24 hr post-CLP: sham

antibody  $82 \pm 14$  ng/ml; anti-CD147 antibody  $16 \pm 7$  ng/ml  $n=8$   $p=0.01$ . Serum IL-10 24 hr post-CLP: sham antibody  $1370 \pm 146$  pg/ml; anti-CD147 antibody  $667 \pm 265$  pg/ml  $n=8$   $p=0.03$ ). **Conclusions** By applying proteomics to a clinically relevant mouse model of sepsis we identified a number of novel proteins that changed in abundance. The inhibition of the receptor for one of these proteins, cyclophilin, attenuated sepsis-induced acute renal failure. The application of proteomics to sepsis research can facilitate the discovery of new therapeutic targets.

### Improvements in Insulin Sensitivity by PPAR $\gamma$ -Agonists are Not Through Inhibition of Glucocorticoid Action in Humans

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**Background** PPAR $\gamma$ -agonists are used to improve insulin sensitivity in patients with type two diabetes mellitus. Recently, in vitro and in vivo work in rodents has shown that PPAR $\gamma$ -agonists downregulate 11 $\beta$ -hydroxysteroid dehydrogenase type 1 (11 $\beta$ -HSD1) in adipose tissue, an enzyme which converts cortisone to cortisol and thus increases local active glucocorticoid (GC) concentrations in adipose tissue and the liver. This study tested whether PPAR $\gamma$ -agonists regulate 11 $\beta$ -HSD1 in humans, and if their insulin-sensitising effect is mediated by glucocorticoid inhibition in adipose tissue. **Methods** 12 healthy men with BMI 20-40 kg/m<sup>2</sup> were recruited into a double-blinded 2 phase crossover study. Subjects were randomised to rosiglitazone 4mg once daily or placebo for 5 weeks. Assessments were performed on days 28 and 35 of each phase, in advance of which they received GC blockade or placebo in randomised order. GC blockade was achieved using RU38486 400mg and metyrapone 1g the night before and the morning of assessments. During assessments, a subcutaneous abdominal adipose tissue biopsy was obtained for mRNA analysis by real time PCR, followed by a 4 hour incremental insulin infusion at rates of 0, 0.01, 0.03 and 0.1 IU/kg/hr, each for 1 h. Bloods were taken during the infusion for insulin, C-peptide, glucose, free fatty acids (FFAs), and glycerol to assess intermediate metabolism. Data are mean  $\pm$  SEM \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$  compared with placebo. **Results** Rosiglitazone did not alter glucose levels or infused insulin concentrations, but decreased C-peptide levels at baseline and during the insulin infusion, consistent with insulin sensitisation by this drug. Rosiglitazone reduced plasma FFAs at baseline and throughout the insulin infusions (\*\*) and reduced plasma glycerol at baseline (\*\*). Plasma cortisol was decreased by 14% (\*) on rosiglitazone but adipose tissue 11 $\beta$ -HSD1, GR $\alpha$ , H6PDH, and adiponectin mRNA levels were no different following treatment. The GC blockade protocol suppressed plasma cortisol by 62% (\*\*\*) and increased plasma ACTH  $\sim$ 5-fold (\*\*). RU38486 was detected at therapeutic concentrations in plasma ( $6.2 \pm 0.6 \mu\text{mol/l}$ ). However GC blockade did not significantly alter the effects of rosiglitazone on insulin sensitivity or adipose gene expression. **Conclusions** This study suggests that glucocorticoid availability does not determine the insulin sensitising effects of PPAR $\gamma$ -agonists in humans, despite lowering circulating cortisol. Unlike in rodents, short-term treatment with rosiglitazone does not alter expression of 11 $\beta$ -HSD1 and thus changes in this enzyme are unlikely to contribute to the therapeutic benefits of thiazolidinediones in humans.

### Scottish Berries - Potential for Dietary Chemoprevention?

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**Background** It has been suggested that diets high in fresh fruit and vegetables are good for our health. Epidemiological evidence supports this idea in relation to prevention of cardiovascular disease. There is, however, little evidence in human studies that proves conclusively the same holds true for the prevention of cancer. In recent years much effort has gone into identifying constituents of fruits that are potentially chemopreventative. The major compounds to demonstrate this property in vitro are the anthocyanins, flavanols and polyphenols. Ellagic acid (EA) is a polyphenol which inhibits neoplastic growth through independent and synergistic mechanisms. EA is produced through enzyme-controlled condensation reactions of hydrolysable ellagitannins (ETs). Of all the fruits investigated, EA- in either its free-state or incorporated in ETs - exists in particularly high concentrations in berries. Our aim was to determine the differential sensitivity of a range of human cancer cells to, initially, EA and thereafter extracts from Scottish berries containing different ETs and concentrations of ETs. Due to technical difficulties in determining the bioavailability of EA in man, the majority of research in this field uses cell culture models. Standard cell culture techniques together with MTT

cytotoxicity, Lowry and Trypan Blue exclusion assays were used to determine cytotoxicity. Cell growth-inhibition was considered in terms of viability, cell number and protein content. All of the human cancer cell lines responded to ellagic acid treatment by decreasing cell growth. The degree to which cell growth is altered is dependent upon a number of factors. It was discovered that with the type of cancer cell under investigation the efficacy of EA and berry extracts alters, producing a spectrum of sensitivity: Non-Small Cell Lung Carcinoma (A549) > Breast Adenocarcinoma (MDA MB 231) > Colorectal Carcinoma (DLD-1). This finding is counter intuitive given that in vivo colorectal cancer cells theoretically have a higher degree of exposure to ETs and bacteria capable of releasing EA. A dose and time-response relationship was clearly demonstrated for all cell lines investigated. DAPI staining was used to morphologically confirm that the cytotoxicity has been brought about by apoptosis. The involvement of apoptosis as a form of controlled cell death implies that EA derived from berries may exert some of its effects by interfering with the polyamine pathway. Likewise, the recent discovery that EA is a potent inhibitor of Organic Anion Transporters (OATs) may have implications for its distribution and bioavailability. Research to date has produced encouraging data and helped towards a better understanding of the benefits in a diet rich with berries.

### Poster Presentations from Scottish Society for Experimental Medicine

The following poster presentations are available at [www.smj.org.uk](http://www.smj.org.uk)

### Isolation Of Endothelial Cell Microparticles From Human Blood For The Discovery Of Biomarkers

F Zhu, S Johnston, D Webb, J Dear

### Circulating Endothelial Cells, Arterial Stiffness and Cardiovascular Mortality Risk Stratification in Hypertension

CJ Boos, M Karpha, DG Beevers and GYH Lip

### Circulating Endothelial Cells As An Index Of Endothelial Dysfunction In Breast Cancer: Relationship To The Nottingham Prognostic Index Scoring System

PKY Goon, PS Stonelake, AD Blann, GYH Lip

### Endothelial Dysfunction in Healthy Smokers and the Effect of Sildenafil Citrate

P Lilitkarnatukul, JJ Oliver, SJ Leslie, K Grainger, G Atkins, J Beveridge, DJ Webb

### Inhalation of Particulate Air Pollution Impairs Vascular Function in Man

NL Mills, AJ Lucking, J Beveridge, L Flint, F Paterson, P Fokkens, NA Boon, A Blomberg, T Sandstrom, K Donaldson, FR Cassee, DE Newby

### Effects of Phosphodiesterase Type 5 Inhibition on Exercise-Induced Vasodilatation in Hypertensive Patients

TM Attinà, LS Malatino, SR Maxwell, PL Padfield, DJ Webb

### The Role of Nitric Oxide and Prostacyclin in the Vascular Effects of Thrombin Receptor Activation in Vivo in Man

IJ Gudmundsdottir, P Dawson, CA Ludlam, KA Fox, DE Newby

### The Role of Nitric Oxide in the Cutaneous Response to Ultraviolet Irradiation - the Story so Far

M Mowbray, R Weller, RE Morris

### Adverse Cardiovascular Effects of Air Pollution in Patients with Coronary Heart Disease

E Vink, NL Mills, H Törnqvist, MC Gonzales, SD Robinson, S Soderberg, NA Boon, W MacNee, K Donaldson

### CT Appearances at Baseline in the MRC Third International Stroke Trial: Relationship with Time from Stroke Onset to Scan and Stroke severity

W Whiteley, P Sandercock, J Wardlaw

### Diffusion Weighted MRI in Patients with Mild Strokes - Factors Associated with Negative DWI and Implications for Trials and Observational Studies

F Doubal, J Wardlaw, M Dennis

**Cellular Localisation of the Endothelin Type B Receptor in the Renal Medulla of Transgenic Mice***D Armour, D Webb, Y Kotelevtsev***Intravascular Glucocorticoid Metabolism by 11 $\beta$ -Hydroxysteroid Dehydrogenase Type 1 Following Arterial Injury***LJ Macdonald, AR Dover, E Miller, BR Walker, PWF Hadoke***Effect of Overweight/Obesity on Efficacy of Assisted Reproductive Techniques within a Tayside Population***G Hancock, V Kay, S Ray***Relationship of BMI to Adipocyte Function in Normal Pregnancy***SS Huda, E K Tan, C Perry, I Greer, N Sattar, D Freeman***Anxiety in Pregnancy: Antenatal Influences and Perinatal Outcomes in the Hospital and Community Setting***F Denison, MD Gunning, CJ Stockley, SP Ho, BR Walker, RM Reynolds***Parallel Developmental Changes in Male and Female Germ Cells in Human Foetal Gonads***G Cowan, N Fulton, S Coutts, R Anderson\*, P Saunders***The Impact of the Revised Alanine Transferase Criteria on the Prevalence and the Pattern of Abnormal Liver Enzymes in Type 2 Diabetes Patients***KA Lockman, NC McAvoy, AJ Jaap, PC Hayes***Are there Multiple Endogenous Glucocorticoids? Chronic Effects of 5 $\alpha$ -Tetrahydrocorticosterone in C57BL/6 Mice***C Yang, DEW Livingstone, AG Rossi, BR Walker, CJ Kenyon, R Andrew***NAFLD in Type 2 Diabetes***KA Lockman, NC McAvoy, AJ Jaap, PC Hayes***11 $\beta$ -Hydroxysteroid Dehydrogenase (EC 1.1.1.146): 1: Key Regulator in Oxysterol Metabolism?***T Mitic, I McNae, SP Webster, M Wamil, BR Walker, PWF Hadoke and R Andrew***Cardiovascular Risk in Type 2 Diabetes is Associated with Glu298Asp Polymorphism of the eNOS Gene: a Go-DARTS Study***V Godfrey, ASF Doney, C N A Palmer, A Whitley, CC Lang***Expression of Functional NMDA Receptors in Human Osteoarthritic Chondrocytes***L Ramage, G Hardingham and D Salter* **$\beta$ 1 - Integrin Regulation of Mechanotransduction in Osteoarthritic Human Articular Chondrocytes***KJ Elliot, DM Salter***A Randomised Controlled Trial of Routine Single or Multiple Dose Superactivated Charcoal for Self-Poisoning in a Region with High Mortality***M Eddleston, A Hittarage, W Dissanayake, S Azber, E Juszczyk, N Buckley, MHR Sheriff, DA Warrell***Divergent Modulation of Apoptotic Pathways in Primary Human Innate Immune Cells by the Cationic Host Defence Peptide LL-37***PG Barlow, Y Li, TS Wilkinson, C Haslett, AJ Simpson, REW Hancock, Donald J Davidson***The Role of Galectin-3 in Acute Bacterial Pneumonia***S Farnworth, N Henderson, A Mackinnon, J Simpson, C Haslett, T Sethi***Hemin Treatment of Murine Macrophages Results in an HO-1 Dependent Anti Inflammatory Phenotype with Reduced Phagocytic Ability***V Ramdas, D Ferenbach, D Walbaum, J Hughes, D Kluth*