arteries and vascular cells from WKY rats were studied. Vascular function was analysed by wire myography in the presence or absence of FetA (50 ng/mL) and/or CLI095 (CLI – 10^{-6}M – TLR4 inhibitor). Reactive oxygen species (ROS) were measured by chemiluminescence, Amplex Red (H_2O_2) and ELISA to nitrotyrosine levels (peroxynitrite – $ONOO^-$). Protein oxidation and levels were measured by immunoblotting. WKY vessels exposed to FetA were less sensitive to acetylcholine (Ach)-induced and sodium nitroprusside (SNP)-induced relaxation, while sensitivity to phenylephrine was increased by FetA; an effect blocked by N-acetylcysteine (antioxidant) and ML171 (Nox1 inhibitor). Inhibition of TLR4 blocked FetA effects on endothelial-dependent relaxation and contraction, but not on endothelial-independent relaxation. FetA increased ROS production (1 fold), but decreased H_2O_2 intracellular levels (0.5 fold); and increased gene levels of IL6 (2 fold), IL1Ã□Â² (1 fold), RANTES (1 fold) and MMP2/9 (2 fold) in endothelial cells (EC) ($p<0.05$); an effect blocked by CLI095. ROS production (0.5 fold), as well as, H_2O_2 (0.5 fold) and $ONOO^-$ (1 fold) levels, were increased by FetA in VSMCs ($p<0.05$). Protein oxidation was also increased by FetA in VSMCs (1 fold, $p<0.05$). In EC, eNOS inactivation (1 fold) and JNK activation (0.5 fold) were increased by FetA ($p<0.05$). In VSMCs, Rho kinase activity was increased (2 fold, $p<0.05$) at 30 min; while myosin light chain (MLC) activation was only increased (0.5 fold) at 15 min. In summary, FetA seems to influence vascular function through Nox1-ROS dependent mechanisms, where only endothelial dysfunction and contractile responses were mediated by TLR4 activation. Identification of FetA as a ligand of TLR4 and further characterisation of FetA-induced signalling may be of importance to the development of new therapy for treatment of hypertension.

171 AMPHIPHYSIN II (BIN1) DRIVEN TRANSVERSE TUBULE FORMATION IN CARDIAC MUSCLE

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Transverse (t)-tubules are vital for maintaining normal contractility of the heart through the tight regulation of excitation coupling. In cardiac disease, such as heart failure, t-tubule loss is closely associated with decreased synchrony of calcium release from the sarcoplasmic reticulum, resulting in impaired contractility. Thus, determining the mechanisms that control t-tubule formation is essential for understanding cardiac disease. Evidence suggests that the protein Amphiphysin II (AmpII) controls t-tubule formation in cardiac muscle and thus, may play a vital role in calcium regulation. Several studies, including our own, have shown that gene silencing of AmpII causes t-tubule loss in both skeletal and cardiac muscle. Furthermore, in non-muscle cells that usually lack t-tubules, expression of some variants of AmpII led to tubule formation. We therefore aimed to extend these observations and determine if AmpII is sufficient to drive t-tubule formation in the heart.

Neonate rat ventricular myocytes (NRVMs) were isolated from 2 day old rats and maintained in culture. Vectors encoding isoforms 5, 8 and 9 of the AmpII gene (Bin1) with a C-terminal mKate2 fluorescent protein tag were transiently expressed in NRVMs using FuGENE 6 lipofection. A vector containing the mKate2 fluorescent tag only was used as negative control. After 48 hours, over-expression of Bin1 was confirmed at both the mRNA and protein level. Tubule formation was assessed using the membrane dye FM-464 and confocal microscopy. Of cells successfully transfected with Bin1, 95% had developed tubule structures. Conversely, tubules were absent in cells only expressing the fluorescent tag ($p<0.001$). Furthermore, Bin1 isoform 8 expression led to formation of more tubule structures when compared to isoform 5 and 9 ($p<0.05$).

To determine if Bin1 driven tubules are functional, transfected cells were loaded with the Ca2+ indicator Fluo-8 AM and field stimulated. When compared with untransfected myocytes, expression of Bin1 isoforms 5, 8 and 9 increased the amplitude of the systolic calcium transient ($p<0.05$). Furthermore, transfection with Bin1 isoforms 5 and 9 led to faster rise and decay of the systolic calcium transient ($p<0.05$). Transfection with the control vector only had no effect on the calcium handling when compared with untransfected cells.

Over-expression of Bin1 isoforms 5, 8 and 9 led to the formation of tubular structures in NRVMs. Whilst Bin1 isoforms 8 appears to play more of a role in tubule formation in NRVMs, these data suggest that other Bin1 isoforms (5 and 9) may enhance calcium kinetics. These data therefore suggest that Bin1 plays a vital role in tubule formation and development in cardiac myocytes. Given the importance of t-tubules to normal excitation contraction coupling and their perturbation in heart failure we therefore suggest that Bin1 might be a novel therapeutic target.

172 β²-CATENIN MEDIATES THE ANTI-APOPTOTIC EFFECTS OF NO IN ENDOTHELIAL CELLS

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Î²-Catenin Mediates the Anti-Apoptotic Effects of NO in Endothelial Cells

Apoptosis is implicated in a number of cardiovascular diseases. Increased endothelial cell apoptosis is associated with the development of atherosclerotic plaques. Strategies to promote endothelial cell survival may therefore represent a novel therapeutic approach in cardiovascular disease.

Nitric oxide (NO) and β²-catenin have both been shown to promote cell survival. Recently we showed that pharmacological activation of the endothelial nitric oxide synthase (eNOS), acting through cGMP, can promote nuclear translocation and transcriptional activity of Î²-catenin.

Using an orbital shaker system to generate shear stress, we investigated the physiological role of β²-catenin as a mediator of NO-induced cell survival in endothelial cells. Human umbilical vein endothelial cells (HUVEC) exposed to flow for 72 hour at 150 rpm on an orbital shaker exhibited different degrees of apoptosis between the undisturbed flow (UF) zone in the periphery of the well and the central disturbed flow (DF) zone in the centre of the well (1.54%±0.26 (mean± SEM) cleaved caspase positive cells in DF zone compared to 0.26%±0.07 in UF zone; $n=3$ $p<0.01$). The degree of apoptosis increased in both UF and DF areas when cells were treated with an inhibitor of beta-catenin transcriptional activity, with 4.45%±0.86 apoptotic cells in the DF zone compared to 0.57%±0.11 in the UF zone ($n=3$ $p<0.05$ for non-treated to inhibitor treated in the DF zone).

We confirmed expression of eNOS and beta-catenin in both UF and DF zones by quantitative-PCR and immunostaining, as well as the interaction between eNOS and beta-catenin in both regions by proximity ligation assay. We then studied the expression of several pro-survival and anti-apoptotic genes by q-PCR in HUVEC exposed to flow for 72 hours. We observed that the expression of Bcl-2 and survivin were downregulated in UF exposed cells compared to static conditions (62% ±0.08 downregulation of Bcl-2% and 67%±0.08 downregulation of survivin, n=3 $p<0.01$ in both cases); and using specific beta-catenin/TCF-LEF inhibitors we identified survivin as an anti-apoptotic gene regulated by beta-catenin (89% ±0.03 downregulation in inhibitor treated samples compared to non-treated, n=3 $p<0.001$) in endothelial cells under flow.

We also investigated the reciprocal effects on eNOS of activation of Wnt signalling and beta-catenin in HUVECs. Using Wnt3a and LiCl, that lead to the accumulation of beta-catenin in the cytoplasm, we found that phosphorylation of eNOS at Ser1177 increased 4 fold after 2–5 min (n=3 $p<0.01$) leading to enzyme activation. Phosphorylation of eNOS at Ser633 and Ser114 was also observed both in HUVEC exposed to UF flow for 72 hour in an orbital shaker and in HUVEC stimulated by Wnt3a.

Together our results indicate that beta-catenin is a key mediator of flow-induced anti-apoptotic effects, both through transcriptional regulation and through activation of eNOS phosphorylation in endothelial cells.

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173 INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN 2 (IGFBP2): A POSITIVE REGULATOR OF ANGIOGENESIS?

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Introduction The insulin-like growth factor binding protein 2 (IGFBP2) has been implicated in the regulation of insulin-like growth factor (IGF) activity in most tissue and organs. IGFBP2 has, however, been reported to have additional intrinsic, IGF independent properties. Low circulating IGFBP2 levels are associated with obesity in humans. High levels of IGFBP2 on the other hand are linked to increased tumour angiogenesis in humans. In this setting increased angiogenesis has been suggested to be caused by indirect rather than direct modulation of endothelial cells. Here we tested the hypothesis that IGFBP2 is able to modulate endothelial cell function directly.

Basic methods and Results: Using immunoblotting, we show that acute stimulation of human umbilical vein endothelial cells (HUVEC) with 15 nM IGFBP2 lead to an increase in phosphorylation of Akt/PKB, an important regulator of endothelial cell function. Data obtained from an *in vitro* model of sprouting angiogenesis suggests that stimulation of HUVEC with IGFBP2 induced endothelial cell sprouting. Mice overexpressing human IGFBP2 showed increased tip cell formation and vascular density in the mouse retina model of developmental angiogenesis.

Conclusions Here we present data from *in vitro* and *in vivo* models of angiogenesis supporting our hypothesis that IGFBP-2 is able to directly modulate endothelial cell function.

174 COMPARISON OF THE MECHANICAL PERFORMANCE OF POLYMERIC AND METALLIC SCAFFOLDS – TESTING AND MODELLING

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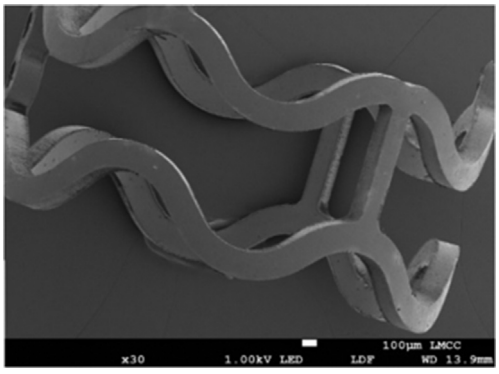
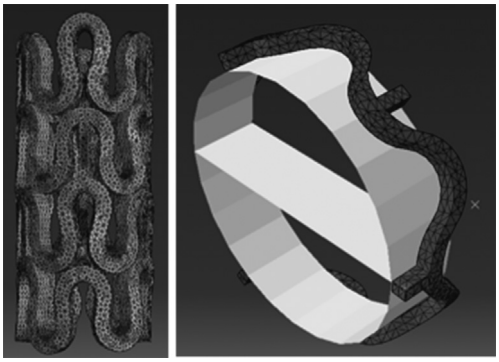
Percutaneous coronary intervention is a standard procedure to resolve blockages within artery, which involves the implantation of stents to maintain vessel patency. Currently, bioresorbable scaffolds (BRSs) are in the process of replacing the metallic permanent predecessor (drug eluting stents) commonly used in stenting. BRSs are commonly made of poly (L) Lactide (PLLA), an aliphatic polyester which is biodegradable and biocompatible with a wide range of medical applications. The performance of these scaffolds is not well defined in comparison to their metallic counterparts.



Abstract 174 Figure 1 SEM image of a polymeric scaffold.

The aim of this project is to assess the mechanical performance of PLLA scaffolds (Figure 1), with a direct comparison to that of metallic stents. This will be achieved through mechanical testing of structural rings at different load rates and ranges. Scaffolds will also be characterised using nano/micro indentation. The results will be used to support computational work for predicting the behaviour of both stents during crimping and expansion (Figure 2).



Abstract 174 Figure 2 Illustration of computational assessments of BRS scaffold performance (ring test and crimping).

Figure 3, Nanoindentation data on BRS.
Figure 4, AFM data on BRS.