tected cells from peroxynitrite injury. In contrast, statins, at doses that caused apoptosis (5.8±0.9% versus control 1.1±0.6%, p=0.003), reduced expression of Hsp75 and SVV, and induced depolarization. Knockdown of Hsp-75 decreased MMP and sensitized VSMC to peroxynitrite, whereas overexpression of Hsp-75 increased MMP and protected cells from mitochondrial depolarization.

**Conclusions:** These data suggest that an organelle-specific Hsp chaperone complex regulates VSMC survival by control of mitochondrial membrane integrity. This cytoprotective mitochondrial network may be a relevant molecular target for modulation of the vascular injury response.

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## PS198.

### Vitamin K2 Reduces Neointimal Hyperplasia and Calcification in a Uraemia Arteriovenous Fistula Rat Model

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**Objectives:** Chronic kidney disease (CKD) triggers the development of neointimal hyperplasia (NIH) and calcification in arteriovenous fistulas (AVF), thus contributing to AVF failure. There is strong evidence that vitamin K-dependent proteins are involved in these pathophysiological events and it has been shown, that CKD patients suffer from vitamin-K2-deficiency. The aim of this study was to assess the impact of a perioperative vitamin K2 administration on AVF remodelling and maturation in a validated uraemia AVF rat model.

**Methods:** CKD was induced with an adenine rich diet. AVFs were microsurgically created in the femoral vessels. Adenine fed animals were either fed with vitamin K2 supplemented food pre- and postoperatively (preventive Group 1) or immediately after the operation (therapeutic Group 2). A third CKD group (Group 3) was postoperatively fed with normal diet. Group 4 was fed preoperatively with normal diet and postoperatively with diet supplemented with vitamin K2. Animals were sacrificed on days 21, 42 and 63 for histological and immunohistochemical analyses of the AVFs and the contralateral femoral vessels.

**Results:** Groups 1 and 2 presented a significant reduction ($p<0.0002$) up to 40% of the NIH formation in the fistula vein, compared to Group 3. Significant has been the reduction of the calcification of Groups 1 and 2 ($p<0.0001$) after administration of vitamin K2, compared to Groups 3 and 4. The shrinking of the media of Groups 1 and 2 because of myofibroblast migration to the intima has been likewise significantly reduced ($p<0.0001$) compared to Group 3 .

**Conclusions:** The results of the current study clearly demonstrate that a preventive or therapeutic vitamin K2 administration, effectively prevents both calcification and neointimal hyperplasia in fistula veins of rats with CKD.

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## PS200.

### Notch Activation Induces Endothelial Cell Senescence and Pro-Inflammatory Response: Implication of Notch Signaling in Atherosclerosis

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**Objectives:** Since Notch is essential to vascular development, we sought to determine the biologic effect of Notch signaling on mature human endothelial cells (EC) and to investigate a potential link between Notch signaling and Atherosclerosis.

**Methods:** Given that EC senescence and vascular inflammation are features of Atherosclerosis, we utilized in vitro loss-and-gain of function approaches to evaluate the role of Notch signaling in inducing secretion of pro-inflammatory cytokines and EC senescence. Human and murine arterial samples were evaluated for Notch expression. Notch gene profile was studied in 1179 human blood samples (from patients concurrently phenotyped for CAD by cardiac catherization). Genotyping was performed using the Affymetrix Genome-Wide Human SNP Array 6.0.

**Results:** The Notch pathway was significantly activated in aged but not young human EC. Enforced Notch signaling activation resulted in EC senescence and induced significantly higher expression of several molecules implicated in the inflammatory response (IL-6, IL-8, IL-1α, RANTES, ICAM-1). Upregulated cytokines were specifically responsible for mediating leukocytes transendothelial migration. Several Notch pathway components were upregulated in EC at atherosclerotic lesions from human and mouse arteries. Genetic association analysis detected significant interactions between Single Nucleotide Polymorphisms (SNPs) in Notch and Notch-target genes [Notch3 x

Hey1, DLL4 x Hes1, and Notch1 x Hes1; ($p < 0.01$)] that may confer CAD risk.

**Conclusions:** The Notch pathway is activated in atherosclerotic plaques and results in endothelial inflammation and senescence. Notch signaling may be linked to CAD risk. These findings implicate, for the first time, a potential involvement of Notch signaling in Atherosclerosis.

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## PS202.

### Elevated Peri-Operative C-Reactive Protein Impedes Late Vein Graft Remodeling

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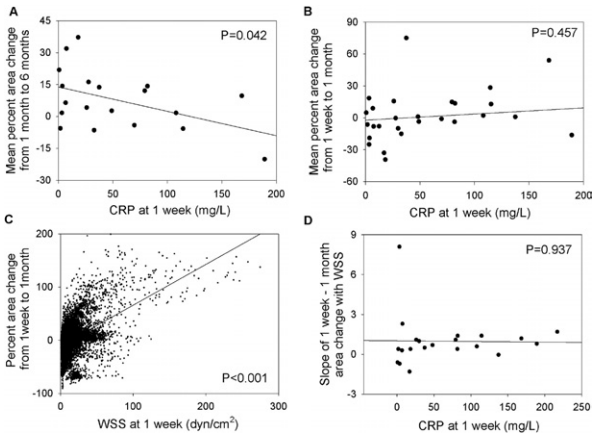
**Objectives:** Enhanced systemic inflammation, as measured by C-reactive protein (CRP), has been associated with reduced early outward remodeling and long-term vein graft (VG) failure. This study seeks to define the long-term changes in VG remodeling that are induced by this pro-inflammatory phenotype.

**Methods:** A prospective study was performed on 31 patients undergoing autogenous VG placement. CT scans, with computational analysis of wall shear stress (WSS), were performed at 1wk, 1m, and 6m to evaluate lumen remodeling. hsCRP was obtained pre-op and 1wk post-op.

**Results:** Late changes in VG lumen diameter (between 1m to 6m) were negatively correlated with CRP levels obtained at 1wk post-op (Fig A, P=0.04), while early changes in VG diameter (1wk and 1m) were independent of 1wk CRP (Fig B, P=NS). Adaptation of the lumen was positively correlated with WSS (Fig C, P<0.001) and was not influenced by the 1wk CRP level (Fig D, P=NS). Neither early or late geometric VG changes nor the adaptive response to WSS were correlated with pre-op CRP levels. Six (of 31) VG were revised or occluded within 1 year, and this was not dependent on either pre-op or 1wk CRP.




**Conclusions:** Late VG remodeling was significantly reduced by an enhanced peri-operative inflammatory response, while early VG adaptation was independent of this response. In contrast to published reports, we found no correlation between the baseline inflammatory state of the patient and WSS-dependent VG adaptation. The current studies suggest long-term structural modulation of the VG by the inflammatory system as the dominant determinant for these events.

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## PS204.

### The Toll-like Receptor 2 Ligand HMGB-1 Contributes to Skeletal Muscle Damage in Critical Limb Ischemia

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**Objectives:** Inflammation and cell damage contribute to the pathophysiology of critical limb ischemia (CLI). Toll-like receptors (TLRs) play an important role in inflammation and tissue damage probably in response to the release of endogenous ligands. We hypothesize that the expression of TLRs and their endogenous ligands is upregulated in ischaemic skeletal muscle with consequent activation of their signaling pathway, which could lead to an increase in inflammatory cytokine release contributing to muscle damage.

**Methods:** TLR expression was studied in ischaemic and control human muscle biopsies and in C2C12 myotubes cultured in ischaemic conditions using RT-PCR and Western blot. Western blot was used to measure the expression of the endogenous ligand, high mobility group box protein-1 (HMGB-1). Functional effects of TLR2 antagonism on ischaemia-induced IL-6 release and cell death were studied by incubating myotubes with neutralizing TLR2 antibody. IL-6 release was assayed by ELISA. Apoptosis was assessed using cleaved caspase-3 and bax/bcl-2 ratio measurements.

**Results:** TLR2 mRNA and protein expression was significantly upregulated in ischaemic muscle and in C2C12 myotubes cultured in ischaemic conditions ($p<0.05$). Raised levels of HMGB1 were demonstrated in ischaemic human muscle biopsies and in ischaemic C2C12 myotubes.TLR2 antagonism reduced ischaemia-induced IL-6 production and apoptosis in culture.