system may thus be important for plaque stabilization. At the same time, the mitogenic activity of IGF-1 can promote development of intimal hyperplasia and restenosis. Aim: To evaluate the possible effect of IGF-1R inhibition on atherosclerosis progression.

Methods: 16 ApoE-/- mice were treated orally with PPP 3.2 mg/day between 11 and 25 or 33 weeks of age. Control group consisted of 16 animals given normal chow diet. Atherosclerosis burden was evaluated by en face Sudan IV staining of aortas. At the same time structure of the atherosclerotic plaques was assessed on the crossections of brachiocephalic trunk.

Results: Animals treated with PPP had decreased plaque area in the aorta; this effect was stronger at 25 weeks of age (5.2% of the aortic wall in control group and 2.2% in PPP group) and less pronounced at 33 weeks of age (9.8% and 11.3% respectively). Size of the lesions in brachiocephalic trunk was also reduced by PPP from 48.2% of cross lumen area in control group to 27.0% in PPP group at 25 weeks and from 61.4% to 46.0% at 33 weeks respectively. Furthermore, PPP treated lesions showed more a prominent fibrous cap, had less amounts of cholesterol and a higher cellularity.

Conclusions: Specific inhibition of IGF-1R suppressed progression of atheroma in ApoE deficient animals. Furthermore treatment with PPP has a stabilizing effect on the advanced lesions in brachiocephalic trunk. Further studies are required to investigate mechanisms of the described effects.

Author Disclosures: **M. Axelson**: Nothing to disclose; **U. Hedin**: Nothing to disclose; **O. Larsson**: Nothing to disclose; **A. Razuvaev**: Nothing to disclose; **J. Roy**: Nothing to disclose.

PS208.

Cytostatic Gene Therapy: RNAi-Mediated Survivin Knockdown Induces Cell Cycle Arrest, Polyploidy, and Reduced Migration of Vascular Smooth Muscle Cells (VS MC)

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Objectives: Survivin (SVV) has been implicated in the development of neointimal hyperplasia. We investigated the effects of RNA-interference (RNAi) mediated SVV knockdown on VS MC phenotype.

Methods: SVV knockdown was achieved by either siRNA or lentiviral transduction of shRNA in primary cultured VS MC from human saphenous vein. Proliferation, cell cycle kinetics, apoptosis and migration were assessed.

Results: RNAi reduced SVV gene expression by qPCR (75%, $p < 0.01$), without loss of cell viability. RNAi treatment increased p53 levels, whereas total cellular SVV levels were variable. Subcellular fractionation revealed the majority of SVV protein in VS MC is mitochondrial. SVV RNAi treatment blocked proliferation in response to serum and PDGF-AB ($p < 0.05$, Fig 1). Cell cycle analysis revealed no evidence of G1/S block, but an increased G2/M fraction (27% SVV siRNA vs 17% control siRNA, $p < 0.05$) consistent with a mitotic defect. In a Transwell assay, SVV knockdown reduced migration to PDGF-AB (74% vs controls, $p < 0.01$), and actin-phalloidin staining revealed disorganized actin filaments and polygonal cell shape. Apoptosis was not induced by SVV RNAi, and sensitivity to apoptotic agonists (staurosporine, cytokines) was unchanged.

Conclusions: RNAi targeting SVV in VS MC leads to cell cycle arrest at G2/M and impaired chemotaxis. Regulation of mitosis and apoptosis in VS MC appears to involve differentially regulated pools of SVV. Treatment of VS MC with SVV RNAi might limit the response to vascular injury without destabilizing the vessel wall.

Author Disclosures: M. S. Conte: Nothing to disclose; **H. Lancero**: Nothing to disclose; **C. S. Nabzdyk**: Nothing to disclose; **K. P. Nguyen**: Nothing to disclose; **S. Salek**: Nothing to disclose.

PS210.

Surgical Restoration of Flow Following Prolonged Ischemia Inhibits Neuromuscular Recovery in a Porcine (Sus Scrofa) Model of Extremity Vascular Injury

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Objectives: Despite advances in revascularization following extremity injury the relationship between time to restoration of flow and functional limb salvage is unknown. The objectives of this study are to describe a large animal model of hind limb ischemia/reperfusion and to define an extremity ischemic threshold from clinically relevant measures of neuromuscular recovery

Methods: Sus scrofa swine (N = 38; weight [kg] ± SD 87 kg ± 6.2) were randomized to iliac artery injury and occlusion for 0 (Control), 1 (1HR), 3 (3HR), or 6 (6HR) hours followed by vessel repair and 14 days of recovery. Two groups underwent iliac artery division with no restoration of flow (Ligation), or exposure without intervention (Sham). A composite physiologic measure of recovery (PMR) was generated to assess group differences across 14-days of survival. PMR included hind limb function (Tarlov score) and electrophysiologic measures (compound muscle action potential (CMAP) amplitude, sensory

nerve action potential (SNAP) amplitude, and nerve conduction velocity (NCV)). These results were correlated with peroneus muscle and peroneal nerve histology.

Results: Baseline physiologic characteristics were similar between groups. Neuromuscular recovery in groups with early restoration of flow (Control, 1HR, 3HR) was similar and nearly complete (92%, 98% and 88% respectively; $p > 0.45$). While recovery was diminished in both 6HR and Ligation; Ligation, rather than repair, exhibited greater recovery (68% vs 53%; $p < 0.05$). These relationships correlated with the pathologic grade of degeneration, necrosis, and fibrosis ($p < 0.05$). Using the PMR, the ischemic threshold of the extremity is reached at 5 hours.

Conclusions: This study reports a novel and translatable animal model of extremity ischemia and reperfusion correlating ischemic time to functional markers of recovery. In this model an ischemic threshold of 5 hours is defined after which Ligation is associated with less irreversible injury than surgical restoration of flow.

Author Disclosures: G. Burkhardt: Nothing to disclose; **J. Cowart**: Nothing to disclose; **S. Gifford**: Nothing to disclose; **L. Jones**: Nothing to disclose; **B. Propper**: Nothing to disclose; **T. E. Rasmussen**: Nothing to disclose; **J. Spencer**: Nothing to disclose; **N. Sumner**: Nothing to disclose; **K. Williams**: Nothing to disclose.

PS212.

Human Adult Stem Cells Restore Endothelial Migratory Dysfunction in a Hypoxic Environment

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Objectives: Adipose-derived stem cells (ASC) injected into the blood stream following an ischemic event promote therapeutic angiogenesis in affected tissues. It has been suggested that the stem cells exert their influence via a paracrine effect on native endothelial cells (EC). Using an in vitro model, we evaluate the effect of ASC co-culture on EC function in a hypoxic environment.

Methods: Confluent monolayers of human EC grown on the bottom of transwell plates were wounded to create an even 5mm defect and cultured in either normoxic (21%) or hypoxic (1%) conditions. Human ASC were co-cultured on the top of the transwell plates to evaluate a paracrine effect. Subsequently, EC migration was determined by measuring the wound size after 3d. Media samples were collected and VEGF concentration measured via ELISA. To confirm mechanism, ECs were treated with recombinant VEGF at various concentrations and migration measured. HIF-1α expression was evaluated in ASC by Western blot.

Results: Hypoxia inhibited EC migration (0.87 mm vs 0.78mm, $p < 0.05$) over 3d. Co-culture of ASC enhanced EC migration in both normoxic (0.87 mm to 0.93mm) and hypoxic (0.78 mm to 1.02mm; $p < 0.05$) environments. Media from co-cultures in hypoxia contained significantly more VEGF (708.3 pg/mL) than normoxic co-cultures (311.2 pg/mL) and EC alone (28.9 pg/mL). The addition of VEGF to wounded EC cultures improved migration, but not to the extent of ASC co-culture. Finally, hypoxia increased levels of HIF-1α in ASC.

Conclusions: These results demonstrate that: 1) ASC restore and enhance endothelial cell migratory function in a hypoxic environment, and 2) the effect is primarily, but not totally, due to secretion of VEGF by ASC in response to hypoxia. These results suggest that hypoxic pre-conditioning of ASC might be of value in enhancing their role in therapeutic angiogenesis to treat ischemic heart or limb conditions.

Author Disclosures: H. Abdollahi: Nothing to disclose; **J. Comeau**: Nothing to disclose; **P. DiMuzio**: Nothing to disclose; **S. Fernandez**: Nothing to disclose; **S. McIlhenny**: Nothing to disclose; **R. Song**: Nothing to disclose; **T. Tulenko**: Nothing to disclose; **P. Zhang**: Nothing to disclose.

PS214.

Estrogen Stimulates the Cellular Processes of Intimal Hyperplasia Development via ERK1/2 Dependent Signaling Cascades

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Objectives: Intimal hyperplasia (IH) occurs more frequently in postmenopausal women taking hormone replacement therapy. Matrix metalloproteinases (MMPs) play an important role in the cellular processes of IH development. We have previously shown estrogen (Est) increases MMP activity in vascular smooth muscle cells (VS MCs). Est has been shown to activate MAPK signaling cascades, and MAPKs are known to regulate MMPs in various cell types. Here we investigated Est-modulated signaling pathways involved in MMP regulation and their downstream effect on the cellular processes of IH.

Methods: Est receptor antagonist ICI182780 (ICI; 5μM), tamoxifen (Tam; 5μM), or ERK1/2 inhibitor UO126 (UO; 10μM) were added 30min prior to 24h or 5-45min Est exposure (50nM), and cells were subjected to Western blot analyses, and zymography, Boyden chamber migration, and MTT proliferation assays.

Results: Est exposure caused activation of ERK1/2 at 30 min in human female VS MCs, with no activation of JNK, p38, or PI3K at any time point (n = 2-3). Est-stimulated MMP enzymatic activity was inhibited by exposure to ICI and Tam. Est increased VS MC migration by 17 ± 2% and proliferation by 12 ± 2% vs control ($p < 0.05$; n = 8). ICI and Tam inhibited Est-stimulated migration and proliferation to near basal levels. UO inhibited Est-stimulated