UNIVERSITY OF OTTAWA
HEART INSTITUTE
INSTITUT DE CARDIOLOGIE
DE L'UNIVERSITÉ D'OTTAWA

# Research Day 2011

## Abstracts Program

**May 5, 2011**
**University of Ottawa Heart Institute**

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# Research Day 2011 Abstracts

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## BASIC SCIENCE ORAL PRESENTATIONS

### O-1
### Autologous Endothelial Progenitor Cells Provide a More Extensive Paracrine Repertoire than Resident Cardiac Stem Cells with Equivalent Angiogenic Potential

**Nicholas Latham (Supervisor: Dr. Darryl R Davis)**

**Background:** Cardiac stem cell (CSC) transplantation improves post infarct function by directly growing new myocardium and by preserving reversibly damaged tissue while recruiting endogenous CSCs. These mechanisms are reminiscent of the well studied bone marrow-derived endothelial progenitor cells (EPCs) that enhance cardiac repair solely through cytokine mediated effects. The relative merits of the CSC/EPC cytokine repertoire has long been an area of speculation with no head to head study ever being performed. To accomplish this, we compared the angiogenic potential and cytokine profile of both cell types to identify potential differences, similarities and synergies.
**Methods/Preliminary Results:** Human atrial appendages and blood samples were obtained from patients undergoing clinically-indicated surgery. Resident CSC cultures were established by plating minced and digested tissue fragments in cardiac explant media. Heterogeneous populations of stem cells spontaneously emigrated from the plated tissue and these cells were harvested using mild trypsinization. EPC cultures were established by Ficoll separation of clinical blood samples with cells harvested one week after plating.
To stimulate growth factor secretion, cells were cultured under hypoxic conditions (1% oxygen) in low-serum (FBS 2.5%) growth factor-free media for 48 hours. Conditioned media was collected and frozen. To provide a relative measure of cell abundance, cells were harvested and lysed for protein quantification. A custom cytokine array (RayBio) was used to screen for 58 pro-angiogenic or cardiomyogenic growth factors. Each location within the array returned a positive signal of varying intensity that corresponded to the concentration of a specific growth factor within the conditioned media. Conditioned media from normal human dermal fibroblasts was used a negative cellular control. As compared to conditioned media from normal human dermal fibroblasts, EPCs provided a more extensive repertoire of growth factors than CSCs (37 vs. 7, $p<0.001$). When compared to EPCs, CSCs released significantly greater amounts of seven growth factors including the pro-angiogenic angiogenin and hepatocyte growth factor. The angiogenic capacity of each cell type was established by culturing human umbilical vein endothelial cells in matrigel (Millipore) with conditioned media. CSCs and EPCs have a similar capacity to promote the growth of tubular networks (22.1±6.1 vs. 24.9±0.2 mm) as compared to dermal fibroblast conditioned media and unconditioned media (14.2 ±4.2 vs. 4.9 ±3.4 mm).**Conclusions:** These results demonstrate that EPCs provide a more extensive paracrine repertoire than CSCs with equivalent effects on angiogenesis. Proliferation stimulated by the modest, yet significant, growth factors released by CSCs may underlie this observation. The striking contrast between these paracrine profiles hints that combination therapy with both autologous cell sources may synergistically enhance the revascularization effects of cell therapy.

### O-2
### Automated Synthesis of a Novel F-18 Labeled Tracer for PET Imaging of AT1 Receptors

**Natasha Arksey (Supervisor: Dr. Jean N. DaSilva and Dr. Rob S. Beanlands)**

**Background:** The aim of this study was to synthesize an F-18 labeled analog of losartan for PET imaging of AT1 receptors via "click chemistry" with 2-[18F]fluoro-3-pent-4-yn-1-yloxypyridine ([18F]FPyKYNE). It has been previously shown that the hydroxyl position of losartan is amenable to addition of large groups while maintaining high affinity for AT1 receptors. Several disease states, including cardiac hypertrophy, ischemic and idiopathic cardiomyopathy, and myocardial infarction, result in upregulation of cardiac AT1 receptors. The ability to detect, and more so quantify, this upregulation is important for both disease management and prevention.
**Methods/Results:** The tetrazole group of losartan was first tritylated. Azidation of the hydroxyl group was performed through the Merck method using diphenylphosphoryl azide (DPPA) and 1,5-diazabicyclo(5.4.0)undec-5-ene (DBU) in 4:1 THF/water (40oC, 16 hr). [18F]FPyKYNE was generated in the first reactor vessel of the TRACERLab FX N Pro module (GE Healthcare) via nucleophilic substitution of the nitro group (145oC, 10 min, DMSO). After transferring the reaction mixture onto a silica cartridge, [18F]FPyKYNE was eluted into the second reactor vessel for conjugation with the azido-modified trityl losartan via the Cu(I)-catalysed variant of the Huisgen 1,3-dipolar cycloaddition in the presence of $CuSO_4$ and Na ascorbate in 5:1 DMSO/$H_2O$ (80oC, 20 min). The trityl group was then cleaved with acid (TFA) to produce the [18F]fluoropyridine analog of losartan. Unlabeled fluoropyridine losartan derivative, intermediates and precursors were characterized by ESI-MS, HPLC, and 1H-NMR. The F-18 labeled product was produced in >5% radiochemical yield (decay corrected) in less than 1 hr. HPLC purification and product formulation are currently being optimized. Analytical HPLC (Phenomenex Luna C(18)2 , 10 M) confirmed product identity by co-injection with the cold standard.
**Conclusions:** A [18F]fluoropyridine losartan derivative was synthesized using a completely automated dual reactor module in high purity and radiochemical yield allowing for in vivo microPET studies. In contrast to the 20 min half life of C-11, the 110 min half-life of F-18 will allow for longer scan times and provide the opportunity for multiple scans per patient. This novel tracer, designed from clinically used losartan, shows high potential for monitoring various cardiovascular diseases through imaging AT1 receptor upregulation.

### O-3
### Development of Reporter Gene PET Imaging Techniques for Long-term Assessment of Transplanted Human Circulating Progenitor Cells

**Yan (Mary) Zhang (Supervisor: Dr. Marc Ruel and Dr. Erik J Suuronen)**

**Background:** Direct cell radiolabeling methods with positron emission tomography (PET) have been used to track transplanted stem cells noninvasively. However, this technique can only monitor cells for several hours due to the short half-life of PET radioisotopes. To further investigate cell fate and function in vivo by PET imaging,

we transduced human circulating progenitor cells (CPCs) with reporter genes for long-term assessment of transplanted cells.
**Methods/Results:** A self-inactivating lentiviral particle, carrying a triple-fusion reporter (TFR) construct consisting of PET (herpes simplex virus type 1 truncated thymidine kinase; HSV1-sr39tk), fluorescence (monomeric red fluorescence protein; mRFP), and bioluminescence (firefly luciferase) reporter genes, was produced by transient co-transfection into 293FT cells with pFUUFRTW, psPAX2, and pMD2.G plasmids, using the calcium-phosphate precipitation method. Human CPCs were transduced with the created viral particle at increasing multiplicities of infection (MOI) on RetroNectin-coated plates. The transduction efficiency was determined by flow cytometry analysis of RFP expression. The mean transduction efficiency was 2.6%, 2.2%, 3.3%, 6.7%, 10.3%, 12.4% and 17.3% at MOIs of 0.1, 0.5, 2.5, 12.5, 25, 50 and 100, respectively. The expression of RFP in CPCs was visualized under the fluorescence microscope. Western blot analysis confirmed HSV1-tk protein expression in transduced CPCs. Both transduced and control CPCs exhibited similar rounded or spindle shapes. No significant difference was observed in cell viability between the transduced CPCs and the untreated controls at the low level of MOIs (=50); however, there was a reduction in transduced CPC viability at the MOI of 100 (66.2±9.5% versus 82.8±10.3%, $p<0.05$). The effect of TFR transduction on cellular functions was evaluated by in vitro assays. The migration potential (66.2±21.3 cells/field of view (FOV)) and capillary tube length of cells (5.6±0.6mm) were not adversely affected by the transduction process compared to the control (61.1±19.6 cells/FOV and 5.7±0.5mm; $p=0.7$ and $p=0.8$, respectively). Fluorescence-activated cell sorting analysis isolated the RFP+ transduced CPCs. After 4 weeks, 80.3±8.4% of the sorted cells continued to express RFP.
**Conclusions:** Quiescent human CPCs transduced with a lentiviral vector show stable expression of the triple reporter genes. The TFR gene approach can be developed for tracking transplanted CPCs, while preserving the cells' morphology, viability, migration and angiogenesis capabilities. This technique may be used for the development of reporter gene PET imaging, to monitor the fate of transplanted cells noninvasively, longitudinally, and quantitatively, and thereby help elucidate the mechanisms and effectiveness of cell-based therapies.

### O-4
### Validation of New Image-Derived Blood Input Function for Mouse Heart Positron Emission Tomography Imaging: Evaluation in the Human Arg302Gln-PRKAG2 Mutation-Induced Metabolic Cardiomyopathy

**Stephanie Thorn (Supervisor: Dr. Jean N. DaSilva and Dr. Michael Gollob)**

**Background:** PET imaging can measure non-invasively the extent and progression of metabolic changes in cardiac disorders commonly investigated using transgenic mouse models. Accurate quantification of a blood time activity curve is highly variable due to myocardium activity spillover and camera spatial resolution. Test-retest studies by our group have a population variability of 74% when using the LV cavity for derivation of the blood input function (IF). This study aimed to assess the population variability and test-retest reproducibility of FDG myocardial uptake using the abdominal vena cava IF. These methods were further evaluated in a transgenic mouse model of the PRKAG2 cardiac syndrome, an autosomal dominant disease that results in excessive cardiac glycogen deposition and hypertrophy.
**Methods:** n=6 normal, fed FVB mice were IV-injected with 10-85 MBq of FDG and PET data was acquired for 60 min. Scans were repeated with the same injected dose and at the same time of day 3 days later. Dynamic images were reconstructed with OSEM3D10 iterations/MAP0 iterations. Semi-automated analysis with FlowQuant© software produced left ventricle (LV) polar maps. Blood curves were derived from ROIs drawn on the abdominal vena cava. The fractional rate of uptake values (Ki) were derived from Patlak analysis. Coefficient of variation was determined for population variability and Bland-Altman analysis was used to measure reproducibility. These methods were then applied to n=5 affected PRKAG2 mice (TGmut) and n=3 wildtype (TGwt) controls.
**Results:** Global LV Ki using the vena cava ROI had a mean ± SD of 1.10 ± 0.22 corresponding to a population variability of 20% respectively. The global difference between test-retest Ki values was 0.03 ± 0.28 with a coefficient of repeatability of 0.55.
In the PRKAG2 mouse model a significant global FDG uptake decrease was found in TGmut mice compared to TGwt controls (p value <0.001). These results correspond to a decrease in overall GLUT4 expression.
**Conclusions:** Measurement of an accurate image-derived blood input function using the mouse LV cavity is inaccurate due to the relative size of the heart and spillover from the myocardium. Manual blood sampling is invasive and limited by total blood volume. The vena cava is easily identifiable with limited spillover from adjacent tissues, allowing accurate assessment of the blood time activity curve for Patlak kinetic modeling of myocardial glucose uptake. Using these methods, we are able to assess myocardial glucose uptake in a mouse model of the PRKAG2 glycogen storage disease.

### O-5
### *In Vivo* Evaluation of Angiogenic Factors in A Collagen-Chitosan Matrix as a Potential Islet Transplant Site

**Joanne McBane (Supervisor: Dr. Erik J. Suuronen)**

**Background:** Islet transplantation for the treatment of type I diabetes often fails due to a lack of proper blood supply to support islet survival at the transplant site. Circulating progenitor cells (CPCs) promote angiogenesis while collagen matrices can enhance cell retention and function of CPCs. We are evaluating the use of a collagen-chitosan scaffold as a possible highly vascularized ectopic site for pancreatic islet transplantation. In the current study, collagen and collagen-chitosan matrices +/- CPCs were tested for their ability to promote pro-angiogenic cytokines in vivo and viability of islets cultured in vitro.
**Methods/Results:** Diabetes was induced in nude mice by a tail vein injection of 220mg/kg streptozotocin (STZ). Diabetes induction was considered successful if blood glucose levels were greater than 10mmol/L at one-week post-injection. Human peripheral blood mononuclear cells were seeded onto fibronectin-coated dishes for 4d to obtain CPCs. Collagen and 10:1 collagen:chitosan matrices +/- CPCs were implanted subcutaneously into the backs of diabetic nude mice 4 weeks after STZ treatment, or non-diabetic controls. After 1 or 2 weeks, implants were removed and analyzed by RayBiotech® mouse cytokine array. Neonatal pig islets were harvested from the pancreas and cultured in islet media,

collagen or collagen-chitosan matrices for up to 7d. Analysis of explants revealed that both matrices promoted cell infiltration. After one week, some differences in cytokine levels were observed between the diabetic and the non-diabetic mice; however the more significant differences were seen between the chitosan and collagen matrices. VCAM-1 levels were higher in collagen-chitosan-only matrices versus collagen-only matrices ($p=0.03$), and VEGF levels were higher for collagen-chitosan-only matrices compared to collagen (+/- CPCs) matrices ($p<0.001$). By two weeks, twenty-one pro-angiogenic cytokines were significantly stimulated in the collagen-chitosan matrix compared to collagen matrices (+/- CPCs; $p<0.05$), including VEGF which has been shown to be important for promoting islet vascularization and function post-transplantation. Pro-angiogenic factors monocyte chemotactic protein-5, eotaxin and keratinocyte chemoattractant were also stimulated in two week explants. In vitro, neonatal islets in the collagen-chitosan matrix expressed equivalent levels of insulin after glucose stimulation compared to controls, suggesting islet viability and function.

**Conclusions:** The collagen-chitosan matrix promotes production/retention of pro-angiogenic cytokines compared to collagen-only matrix, which may contribute to the increased vascularization observed in vivo using these matrices. Collagen-chitosan matrices also support islet viability and function. Therefore, the collagen-chitosan matrix warrants further evaluation in islet transplantation models.

### O-6
### Functional Analysis of the Trib1 Locus in Coronary Artery Disease

**Adrianna Douvris (Supervisor: Dr. Ruth McPherson)**

Genome-wide association studies (GWAS) have identified several common genetic variants associated with CAD and CAD risk factors. The TRIB1 locus (8q24.13) is a novel locus associated with plasma TGs, LDL-c, and CAD risk. As part of the Ottawa Heart Study, we have demonstrated that the relationship of this locus to CAD risk is entirely mediated by effects on plasma lipids. Trib1 is a regulator of MAPK activity, and recently, it has been has been shown that Trib1 also regulates hepatic lipogenesis and VLDL production in mouse models. However, the functional relationship between common single nucleotide polymorphisms (SNPs) at the TRIB1 locus and plasma lipid traits is unknown. Specifically, the TRIB1 locus as identified by GWAS comprises a cluster of SNPs significant for TGs, LDL-c, and CAD within an intergenic region 25kb to 50kb downstream of the TRIB1 coding region. By phylogenetic footprinting analysis, we identified an evolutionarily conserved region (CNS1) within the risk locus. DNA sequencing revealed that this region harbors two common SNPs in tight linkage dysequilibrium with GWAS risk SNPs and that also associate significantly with CAD. We investigated the regulatory potential of CNS1 using various luciferase reporter assays in HepG2 cells and demonstrate that this region has promoter activity. Furthermore, a database search for ESTs within the risk locus revealed an EST directly downstream of, and potentially regulated by CNS1. We performed 3'/5' RACE using HepG2 RNA and identified multiple variants of this EST. Consequently, to determine the function of this RNA, we performed siRNA targeting of all variants in HepG2 cells. Our findings demonstrate that knockdown of this EST results in decreased fatty acid synthase (FAS) and ApoB mRNA levels, thereby suggesting that this RNA has a role in the regulation of hepatic lipogenesis. We hypothesize that this EST is a long non-coding RNA since it lacks any significant ORF. Given that it is an intergenic RNA, and has proximity to TRIB1, which has been shown to regulate hepatic lipogenesis, we hypothesize that this RNA may function to regulate the expression of the neighboring genes, thus constituting a link between the intergenic GWAS risk locus, plasma lipids, and CAD.

### O-7
### Role of Glyoxalase-1 in Defective Ischemia-Induced Neovascularization in Diabetes

**Branka Vulesevic (Supervisor: Dr. Erik J. Suuronen)**

**Background:** The risk of atherosclerotic vascular disease and its manifestations is markedly increased in diabetics. Decreased vascularity and perfusion leads to functional ischemia and impaired wound healing. Ischemia-driven neovascularization is defective in diabetes, which can be attributable to endothelial progenitor cell (EPC) dysfunction. Therefore, a better understanding of the mechanisms that control EPCs and blood vessel formation is needed. This study examines the role of the glyoxalase-1 (GLO1)-methylglyoxal pathway in regulating EPC function. In diabetes, methylglyoxal accumulation impairs hypoxia inducible factor-1 (HIF-1) function, needed for neovascularization. It is hoped that increased GLO1, which metabolizes methylglyoxal, can restore EPC function in diabetes.

**Methods/Results:** For this study, we used a transgenic mouse (hGlo1+/-) developed on a C57/BL6 background to over-expresses GLO1. hGlo1+/- and non-transgenic littermates (8-10wk males) were irradiated and transplanted with bone marrow(BM) from enhanced green fluorescent protein (eGFP+) or eGFP/hGLO1+/- donor mice. Four groups were studied:1) wild-type mice receiving eGFP BM; 2) hGLO1+/- mice receiving eGFP BM; 3) wild-type mice receiving eGFP/hGLO1+/- BM; and 4) hGLO1+/- mice receiving eGFP/hGLO1+/- BM. As a control, non-diabetic wild-type mice receiving eGFP donor marrow were used. After marrow reconstitution, mice received streptozotocin for 5 days to induce diabetes. Four weeks later, hindlimb ischemia was induced by left femoral artery ligation. Over 2 weeks post-ligation, blood samples were taken for GFP+ analysis by flow cytometry, and laser Doppler perfusion analysis was performed. Histological sections were analyzed for GFP+ cells and vascular density. A reduced number of circulating CXCR4+ and flk-1+ cells ($0.8\pm0.1$ and $0.3\pm0.1$ fold difference) was observed in diabetic wildtype mice at baseline (versus non-diabetics, $p<0.05$), and these numbers were restored in GLO1 over-expressing mice ($1.4\pm0.3$). In response to ischemia, diabetes reduced the mobilization of GFP+CXCR4+ cells (1.1- to 2.1-foldversus baseline), compared to animals that were reconstituted with hGlo1+/- BM (1.9- to 4.3-fold; $p<0.05$). Vascular density in ischemic hindlimbs was greater in hGlo1+/- BM-reconstituted mice compared to mice with wild-type BM, as determined by staining for von Willebrand factor (endothelial marker) and a-smooth muscle actin (for arterioles). In addition, the reduced perfusion (ischemic/non-ischemic ratio) seen in diabetics after 2 weeks ($0.7\pm0.1$) was restored in both hGlo1+/- mice and in mice that were reconstituted with hGlo1+/- BM ($0.9\pm0.1$ to$1.2\pm0.0$, $p<0.05$).

**Conclusions:** Diabetes reduced the basal numbers and ischemia-induced mobilization of circulating CXCR4+ and flk-1+ cells, which

were restored in Glo1+/- transgenic animals. Two weeks post-ischemia, vascular density and perfusion was greater in hindlimbs of diabetic mice reconstituted with Glo1+/- BM, compared to hindlimbs of diabetic nontransgenic littermates. These data support the concept that GLO1 and its substrate methylglyoxal are involved in regulating EPCs, and that GLO1 over-expression can rescue the defects in EPC mobilization and neovascularization associated with diabetes.

### O-8
### Autophagy and Lysosomal Acid Lipase Regulate Macrophage Reverse Cholesterol Transport

Mireille Ouimet (Supervisor: Dr. Yves Marcel)

**Background:** Promoting cholesterol efflux from macrophage foam cells is an attractive means to treat atherosclerosis. Understanding how cholesterol is mobilized from the lipid droplet (LD), the major site for cholesterol storage, into the reverse cholesterol transport (RCT) pathway is important to devise strategies to enhance cholesterol removal from atherosclerotic plaques. Whereas the current paradigm for macrophage cholesteryl ester (CE) hydrolysis is centered on the activity of neutral lipases, we hypothesized that lysosomal acid lipase (LAL) also contributes to LD CE hydrolysis and that autophagy might be regulating this process.
**Methods/Results:** In cholesterol-loaded murine bone marrow-derived macrophages, microscopy and western blot analysis revealed the co-localization of LDs with autophagosomes. Cytoplasmic LDs, delivered to the lysosomal lumen via autophagosomes, are hydrolyzed by LAL; inhibition of LAL was found to reduce CE hydrolysis and cholesterol efflux. Thus, following LAL-mediated LD CE hydrolysis, the resulting free cholesterol is effluxed to cholesterol acceptors such as lipid-poor apolipoprotein A-I (apoA-I) or high density lipoprotein (HDL). In vitro, autophagy was specifically induced in response to atherogenic lipoproteins, as measured by an upregulation in the autophagy marker microtubule-associated protein 1A/1B light chain 3 (LC3). Interestingly, autophagy was also induced in vivo in peritoneal macrophages from apoe-/- hypercholesterolemic mice as compared to their wild-type counterparts. Whereas efflux to lipid-poor apoA-I is nearly abolished in autophagy-deficient (atg5-/-) macrophages, efflux to HDL was modestly reduced. Hence, autophagy-mediated cholesterol efflux appears to be an ATP-binding cassette A1 (ABCA1)-dependent process. Finally, we show that atg5-/- macrophages have an impaired ability to clear accumulated 3H-cholesterol into the RCT pathway in vivo, highlighting the importance of autophagy-mediated LD catabolism in whole-body RCT.
**Conclusions:** We have uncovered a previously unassigned role for autophagy in the clearance of macrophage LDs. Autophagy therefore represents a novel and potentially interesting target to enhance macrophage cholesterol efflux and promote RCT.

### O-9
### Association of PCSK9 with Low-Density Lipoproteins in Human Plasma

Mia Golder (Supervisor: Dr. Thomas Lagace)

**Background:** Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a secreted serine protease that binds to cell surface low-density lipoprotein (LDL) receptors and mediates their degradation in liver. PCSK9 is abundant in human plasma (30-3000 ng/mL) and its levels are positively correlated with LDL-cholesterol, a major risk factor of cardiovascular disease. Size fractionation studies have shown that circulating PCSK9 displays considerable size heterogeneity due to partial association with undefined high-molecular-weight complexes. In the current study, we have investigated whether PCSK9 is associated with lipoproteins in human plasma.
**Methods/Results:** Using Optiprep density gradient separation of human plasma samples, we show that a subset of PCSK9 is present in highly purified LDL fractions. PCSK9 distribution was increased in the LDL-containing fraction in plasma from patients with familial hypercholesterolemia and thus highly elevated LDL-cholesterol levels. In vitro binding studies showed a direct association between isolated LDL (density 1.019-1.063 g/mL) and fluorophore-labeled recombinant PCSK9, as evidenced by their co-migration in agarose gel electrophoresis. This interaction was highly specific, as it was competed >95% by excess unlabeled PCSK9. Homologous competition binding curves were consistent with a one-site binding model, suggesting a protein-protein interaction involving the apoB100 component of LDL.
**Conclusions:** The association of PCSK9 with circulating LDL particles may affect the ability of PCSK9 to mediate liver LDL receptor degradation.

### O-10
### Nucleotides Regulate Hepatic Lipoprotein Secretion Through Autophagic and Proteasomal Degradation Pathways

Cynthia Chatterjee (Supervisor: Dr. Daniel L. Sparks)

**Background:** Elevations in blood glucose are associated with increased circulating nucleotide levels and abnormal plasma lipoprotein metabolism. Experiments were therefore performed to evaluate how extracellular nucleotides may act to perturb lipoprotein metabolism by affecting hepatic apoA-I and apoB100 secretion.
**Methods/Results:** Adenosine diphosphate (ADP) (20-100 µM) blocks apoA-I secretion from the HepG2 human hepatocyte cell line, but stimulates apoB100 secretion at both 4h and 24h. Dilinoleoyl phosphatidylcholine (DLPC) (12 µM) stimulates a 3-fold increase in apoA-I secretion, which is completely blocked by ADP (100 µM). Conversely, ADP stimulates a 2-fold increase in apoB100 secretion, which is completely blocked by DLPC. ADP affects apoB100 and apoA-I secretion at 4h similar to that observed with proteasomal inhibitors (ALLN and MG132). Proteasomal inhibitors are known to stimulate apoB100 secretion and to promote cellular autophagy. ADP also stimulates autophagy in liver cells and significantly augments a serum starvation-induced autophagic response. ADP increases the levels of the autophagic marker protein, LC3-II, over a 6h time course, relative to that observed for control cells. DLPC, on the other hand, completely blocks the autophagic response and maintains LC3-II levels at a basal state. ADP appears to stimulate autophagy through the classical Akt-mTOR signaling pathway, by blocking the phosphorylation of both Akt and mTOR. An increase in LC3-II levels at 4h is associated with a decrease in both p-Akt and p-mTOR levels. ADP acts through the purinergic receptor, P2Y13 to regulate autophagy and lipoprotein secretion. Knockdown of P2Y13 receptor expression by 50% using siRNA causes a 3-fold increase in apoA-I secretion and triples the DLPC-induction in apoA-I secretion. P2Y13

knockdown decreases LC3-II levels and is associated with an increase in p-Akt compared to control siRNA.
**Conclusions:** These data show that ADP acts through P2Y13 to regulate hepatic lipoprotein secretion by controlling both cellular autophagic and proteasomal degradation pathways. Elevated circulating nucleotide levels in insulin resistance may therefore stimulate apoB100 secretion and in parallel, block apoA-I secretion from liver cells. This may result in decreased plasma HDL / LDL levels, which is a common phenotype observed in patients with elevated blood glucose levels.

### O-11
### Phosphatidylcholine Metabolism Affects Trafficking of LDL-derived Free Cholesterol in Cholesterol-loaded CHO Cells

**Chandra Landry (Supervisor: Dr. Thomas Lagace)**

**Background:** In vitro studies have shown that phosphatidylcholine (PC), the most abundant phospholipid in cell membranes, can positively influence the incorporation and bilateral movement of cholesterol in artificial membrane systems. The potential influence of PC on the cellular trafficking of LDL-derived free cholesterol was examined in sterol regulatory-defective (SRD)-4 cells, a line of chemically mutagenized Chinese hamster ovary (CHO) cells that overproduce cholesterol and fatty acids and are unable to esterify free cholesterol for storage in cytosolic lipid droplets. As a result, these cells accumulate free cholesterol in cellular membranes. Biosynthesis of PC is also elevated in SRD-4 cells due to increased production of a fatty acid-derived activator of CCTalpha, the rate-limiting enzyme in the CDP-choline pathway. However, this increased PC synthesis is balanced by increased catabolism, resulting in minimal net change in cellular PC content.
**Methods/Results:** Incubation of SRD-4 cells with 50 ug/ml low-density lipoprotein (LDL) for 18 h resulted in lysosomal/late endosomal accumulation of free cholesterol as revealed by filipin staining, characteristic of cholesterol trafficking defects seen in Niemann-Pick type C disease. Lysosomal accumulation of LDL-derived free cholesterol was prevented in SRD-4 cells supplemented with lyso-PC (50 uM), a substrate for PC synthesis through the reacylation pathway, and also in cells treated with bromoenol lactone (BEL), an inhibitor of phospholipase A2 implicated in bulk PC turnover. In a counter study, lysosomal cholesterol accumulation in LDL-treated CHO cells was induced using R-propranolol, which inhibits the conversion of phosphatidic acid to diacylglycerol (DAG), a substrate in the CDP-choline pathway. This blockage was also relieved through co-treatment with lyso-PC, suggesting that the mechanism requires intact PC or lyso-PC molecules.
**Conclusions:** These studies support that PC levels in downstream organellar membranes can influence cholesterol trafficking out of the lysosomal compartment.

### O-12
### An integrin-linked kinase mechanism is associated with improved myocardial perfusion, viability and function in infarcted mouse hearts after collagen matrix-enhanced cell therapy

**Ali Ahmadi (Supervisor: Dr. Erik J. Suuronen and Dr. Marc Ruel)**

**Background:** Integrin-linked kinase (ILK) is involved in pathways for cell survival and for the regulation of angiogenic factors. We previously demonstrated that ILK is upregulated in hypoxic circulating progenitor cells (CPCs) upon adhesion to a collagen substrate. In this study, we tested the echo-guided intramyocardial delivery of CPCs, with and without a collagen matrix, in a mouse model of myocardial infarction (MI). We investigated the potential role of ILK and the effect of the collagen matrix on enhancing the therapeutic benefits of CPCs in terms of cardiac function, viability, and perfusion.
**Methods/Results:** Seven days after left anterior descending coronary artery ligation in C57BL6/J mice, animals were randomly allocated to receive echo-guided intramyocardial injection of: CPCs (n=29), matrix alone (n=19), CPCs+matrix (n=29), or PBS (n=15). CPCs were green fluorescent protein (GFP)+ marrow derived cells from C57BL/6-Tg(CAG-EGFP)1Osb/J male mice. 13N-ammonia and 18F-FDG PET imaging, as well as echocardiography, were performed at baseline and 3 weeks after treatment. Hearts were also collected for immunohistochemistry (transplanted cell retention, LV mass preservation, and arteriole density) and Western Blot (ILK expression) analysis. Post-MI baseline ejection fraction (EF) was equivalent in all groups (pooled average=37%). Follow-up EF was significantly greater in the CPC+matrix group (56±2%) compared to CPC (40±2%), matrix-only (36±3%) and PBS (28±2%) groups ($p<0.001$). PET analysis showed improved viability and perfusion (by 35% and 29%, respectively; $p=0.05$) only after treatment with CPC+matrix. Histology showed an anterior to posterior LV wall thickness ratio of 0.66±0.05 in CPC+matrix group, which was significantly greater than for all other groups ($p<0.001$). More arterioles were detected in hearts injected with CPC+matrix (10.9±1.1 per field of view) compared to the other treatments ($p<0.001$). Moreover, there was higher intramyocardial retention of GFP+ cells co-expressing von Willebrand factor in CPC+matrix group (by 3.3-fold), compared to the CPC group ($p=0.001$). ILK expression was higher in hearts treated with CPC+matrix (1.42±0.10 fold) or matrix (1.57±0.07 fold) compared to hearts treated with CPCs-only or PBS ($p=0.02$).
**Conclusions:** A collagen matrix enhances transplanted cell retention, preserves LV wall mass, and improves myocardial viability, perfusion and function, at least in part via an ILK mechanism, thus demonstrating promise as a strategy to stimulate greater cardiac repair with cell therapy.

## CLINICAL SCIENCE ORAL PRESENTATIONS

### O-13
### OPCAB Does Not Preserve Renal Function Better than CABG: Results of a Case-Matched Study in 5,940 patients.

**Elsayed Elmistekawy (Supervisor: Dr Marc Ruel)**

**Background:** Controversy exist regarding the perioperative renal effects of off-pump coronary artery bypass grafting (OPCAB) versus on-pump coronary artery bypass grafting (CABG). Studies have shown conflicting results; however, no large case-matched or randomized comparison has yet been made available in the literature. This study focuses on this clinical controversy.

**Methods:** We studied 5,589 consecutive patients from a single center who underwent OPCAB or CABG between 2002 and 2010. All preoperative, intraoperative, and postoperative data were prospectively collected for all patients. Patients were matched by using a nearest neighbor matching estimation method for average treatment effects, with bias correction (Stata 10.1, College Station, TX). The matching characteristics were: preoperative creatinine, age, gender, body mass index, cerebrovascular disease, peripheral vascular disease, left ventricular grade, diabetes, hypertension, operative priority, and Cardiac Anesthesia Risk Evaluation score.
**Results:** The mean patient age was 64.9 ± 10.0 years, and there were 4,387 (78.5%) males. Mean preoperative serum creatinine clearance was 82.0 ± 32.6 mL/min. Perioperative mortality amounted to 1.45% with OPCAB and 1.73% with CABG (P=0.6). The mean change in creatinine clearance, from preoperative to lowest postoperative value, was -6.3 ± 14.1 mL/min with OPCAB, versus -5.0 ± 15.5 mL/min with CABG (P=0.06). Requirements for de novo postoperative dialysis were equivalent, at 2.6% in OPCAB patients, versus 2.1% in CABG patients (P=0.5). Median postoperative hospital length of stay was 8 days in both groups (P=0.8).
**Conclusions:** OPCAB does not preserve renal function to a greater extent than CABG. In fact, a trend to the reverse exists; however, with no clinically harmful effects.

### O-14
### Prognostic Assessment of Coronary Artery By-Pass Patients with 64-slice CT Angiography: Anatomical Information is Incremental to Clinical Risk Prediction.

**Gary Small (Supervisor: Dr. Benjamin J.W. Chow)**

**Background:** Prognostication in CABG patients can be difficult. Anatomical assessment of native coronary artery disease and graft patency may provide useful information, but the utility of CCTA in the assessment of CABG patients is unknown. We sought to determine the incremental prognostic value of 64 multi-slice coronary computed tomography angiography (CCTA) in coronary artery bypass (CABG) patients.
**Methods:** 657 CABG patients with all cause mortality follow up were identified from the CONFIRM registry, a database of 27,125 patients from 12 multinational CCTA centres. Clinical risk was profiled with NCEP/ATP III guidelines and EuroSCORE. CCTA defined coronary anatomy. Patients were classified by unprotected coronary territory (UCT), or a summary of native vessel disease and graft patency: the coronary artery protection score (CAPS).
**Results:** 76.6% of patients were male and the median age was 68 years. 44 deaths occurred over 48 months follow-up. LVEF, creatinine, age, severity of native vessel disease, UCT, CAPS and EuroSCORE were univariate predictors of mortality ($p<0.001$), NCEP did not predict all cause death ($p=0.27$). In multivariate analysis using EuroSCORE, UCT ($p=0.004$) and CAPS were predictive of events ($p<0.001$). In comparison to EuroSCORE, CAPS score was associated with a 27% net reclassification index.
**Conclusions:** CCTA provides incremental anatomical data to clinical risk assessment to better determine the prognosis of symptomatic patients post CABG. CAPS evaluation using CCTA may help determine those patients at highest risk.

### O-15
### Role of FDG-PET in Imaging of Carotid Atherosclerotic Plaque (FDG PET substudy of the CAIN II Project)

**George Youssef (Supervisor: Dr. Robert S. Beanlands)**

**Background:** Stroke is the third leading cause of death in US and Canada. Vulnerable plaques are identified by abundance of inflammatory cells leading to plaque rupture. Current imaging techniques provide anatomic data but do not indicate plaque metabolic activity. [18F]-fluorodeoxyglucose (18FDG) is a glucose analogue that can be used to image inflammatory cell activity non-invasively by PET. In this study, plaque inflammation was quantified before carotid endarterectomy (CEA) using the combination of 18FDG PET and CT carotid angiography (CTA).
**Methods:** Eleven patients with significant internal carotid artery stenoses (>70%) awaiting CEA were imaged using 18FDG-PET and co-registered CT angiography (CTA). All patients fasted overnight and the images were acquired 3 hours after FDG injection (5 MBq/kg), after which carotid plaques FDG uptake were determined using the Target to Blood ratios (TBR) of standardized uptake values (SUVs) in the plaque and blood pool activity in the internal jugular vein respectively.
**Results:** 11 patients (9 males) with mean age of 66.2 ±9.9 years were studied; 8 patients were symptomatic of which one had bilateral symptomatic significant carotid lesions. The mean symptoms-to-PET scan duration was 97.4 ±99.3 days. TBRs were significantly higher in symptomatic carotids (n=9, mean 4.27±1.14) compared to asymptomatic ones (n=13, mean 2.83±1.06) with P value =0.007. Likewise, when we compared TBRs in carotids with significant stenoses (>70%) (n=12, mean 3.97±1.25) to less severe ones (n=10, mean 2.76±1.03) with P value =0.024.
**Conclusions:** FDG uptake was higher in symptomatic vs asymptomatic plaques and in more severely stenotic lesions. This suggests 18FDG-PET may have a potential role in imaging of inflammation as a marker of instability in carotid atherosclerotic plaques. Further large scale clinical and histopathological studies are required.

### O-16
### A Single CT Study for Attenuation Correction of Rest and Stress SPECT Myocardial Perfusion Images

**Mikael Trottier (Supervisor: Dr. Terrence D. Ruddy)**

**Background:** Advances in nuclear medicine technology have led to cameras that combine CT with SPECT imaging capabilities. These SPECT/CT systems now permit registration of a low dose CT with a SPECT myocardial perfusion scan to correct for attenuation. Attenuation correction (AC) improves the sensitivity, specificity and accuracy of the test, but slightly increases the radiation dose to the patient. To reduce radiation exposure, we evaluated the possibility of doing a single CT after the stress and use the same CT to correct the rest study instead of the standard practice of obtaining a separate CT at both stress and rest.
**Methods:** Image Acquisition. Images were retrospectively examined the rest/stress studies of 150 patients done on an Infinia Hawkeye 4 camera (GE Healthcare). The dataset consisted of 99mTc-Myoview SPECT scans (150 rest and 150 stress) obtained between December 2008 and February 2011. All studies were performed according to

standard clinical imaging protocols in accordance with the American Society of Nuclear Cardiology guidelines with a CT scan obtained at both rest and stress. These studies were reconstructed with OSEM using the rest CT with the rest perfusion scan and the stress CT with stress perfusion scan. These studies were then reprocessed with the use of only the stress CT for the attenuation correction of both stress and rest perfusion scans. The CT image used for AC was aligned manually. Images were blindly evaluated by two independent trained readers. Images were scored using summed stress and rest scores with a 17 segment model and classified as clinically normal or abnormal.
**Results:** Preliminary results on 215 of 300 scans indicate an excellent intra-class correlation coefficient of 0.9/ 0.8 for the summed stress/rest scores and a high concordance of 91% for the clinical evaluation.
**Conclusions:** A single CT scan at stress provides similar attenuation-correction diagnostic accuracy as compared to the standard practice of separately acquired rest and stress CT scans.

### O-17
### Radial versus Femoral Artery Approach for Coronary Angiography and Percutaneous Coronary Intervention in the Extremely Obese

**Benjamin Hibbert (Supervisor: Dr. Edward O'Brien)**

**Objective:** To compare the safety and efficacy of radial versus femoral approach for coronary angiography and PCI in patients with a body mass index (BMI) = 40 kg/m2.
**Background:** Coronary angiography is most commonly performed via femoral artery access; however, the optimal approach in extremely obese (EO) patients remains unclear.
**Methods:** Between January 2007 and August 2010, a cohort of consecutive EO patients who underwent coronary angiography was identified in our center's registry of angiography and PCI procedures. Of 21,103 procedures, 564 (2.7%) were performed in unique EO patients: 203 (36%) via the radial approach and 361 (64%) via the femoral approach.
**Results:** The primary outcome, a combined endpoint of major bleeding, access site injury, and procedural complications occurred in 7.5% of the femoral group and 2.0% of the radial group (OR 0.30 95% CI 0.10 - 0.88, p=0.029), an endpoint driven by reductions in major bleeding (3.3% vs 0.0%, OR 0.12 95% CI 0-0.71, p=0.015) as well as access site injuries (4.7% vs 0.0%, OR 0.081 95% CI 0 - 0.48, p=0.002). There were no differences in procedural complications (1.7% vs 2.0%, OR 1.50 95% CI 0.41- 5.55) but radial access procedures were associated with an increase in procedure and fluoroscopy time when compared to the femoral group.
**Conclusions:** Femoral access for coronary angiography and PCI was associated with more bleeding and access site complications when compared to a radial approach. Important reductions in procedural associated morbidity may be possible with a radial artery approach in EO patients.

### O-18
### Impact of Technetium Shortage on Downstream Utilization of Cardiac Diagnostic Techniques

**Gary Small (Supervisor: Dr. Benjamin J.W. Chow)**

**Background:** In May 2009 the Chalk River nuclear reactor was closed down and dramatically reduced the availability of technetium for medical imaging. In cardiology, thallium was used as an alternative radiotracer in many centers including this institution. The images obtained by thallium are not as count rich as technetium pictures and are consequently recognized to be of a poorer quality. As a consequence thallium imaging may promote further diagnostic investigation either as a result of less reliance on the results or due to false positive findings.
**Methods:** We hypothesized that during the technetium crisis there was an increase in downstream cardiac diagnostic imaging as a consequence of the adoption of thallium as a technetium substitute. We performed a retrospective study of 6000 patients attending for nuclear perfusion studies at a single institution. 3000 patients had been given technetium from May 2008 to March 2009. 3000 patients received thallium during the period of technetium shortage (May 2009- May 2010). Using patient records we determined the number of patients who in the six months following their nuclear study attended for cardiac catheterization or further non invasive imaging test to investigate coronary artery disease (cardiac CT, stress/rest PET, stress echocardiogram).
**Results:** 639 patients in the thallium cohort received addition investigations compared to 436 in the technetium group. There was a 70% relative increase in cardiac catheterizations (404 thallium patients versus 249 technetium patients) and 44% increase in cardiac CT scans (180 versus 125 for thallium and technetium patients respectively). Similar numbers of stress echocardiograms and stress/rest PET studies were performed in each group. Overall there was a 47% increase in downstream cardiac diagnostic testing following the introduction of thallium as a technetium substitute.
**Conclusions**: Should it be shown that as a result of thallium, extra investigation, radiation exposure or clinical risk occurred during the technetium crisis then it might be argued that in future technetium crises, other solutions should be used. In such circumstances implementation of more reliable non-invasive imaging modalities such as cardiac CT or cardiac PET may help to prevent unnecessary utilization of clinical resources, reduce patient radiation exposure and lessen clinical risks from invasive testing.

### O-19
### The Value of Risk Algorithms in Predicting Outcomes for Octogenarians Undergoing Aortic Valve Replacement with or without CABG

**Elsayed Elmistekawy (Supervisor: Dr. Khan Lam)**

**Background:** Aortic valve replacement (AVR) and AVR with coronary bypass surgery (AVR/CABG) are increasingly performed in octogenarians. Assessment of risk based on predictive algorithms could preclude some octogenarians from the benefits of conventional therapy. The objective of this study was to determine the predictive value of risk algorithms on early and late outcomes in this select group of patients.
**Methods:** Between 1999 and 2009, 394 octogenarians underwent AVR (178, 45%) or AVR/CABG (216, 55%) at our institution.
Mean age was 83 +/- 3 yrs; 209 (53%) were male and 388 (98%) received a bioprosthesis for predominantly aortic stenosis (385, 97%). The expected hospital mortality was calculated using the STS predictive risk of mortality (STS) and Logistic EuroSCORE (LES)

algorithms. The STS and LES scores were further divided into low (STS<5.0%, LES <10%), medium (STS 5-10%, LES 11-20%) and high (STS>10%, LES>20%) risk groups. Mean follow-up was 4.7 yrs (range 0.8-11.4, 1699 patient-years) and complete. Parametric and non-parametric analyses were used to determine predictors of outcomes. Observed over expected (O/E) ratios were calculated.
**Results:** Hospital mortality was 32 of 395 (8.1%, AVR 7.3%, AVR/CABG 8.8%, P=.5). Mean expected mortality was 6.5% (STS, O/E=1.25) and 14.3% (LES, O/E=0.56). Mean STS expected mortality in low, medium and high risk patients was respectively 3.3% (O/E=2.3), 6.8% (O/E=1.0) and 14.6% (O/E=0.76); mean expected LES mortality in low, medium and high risk was respectively 8.1% (O/E=0.61), 14.3% (O/E=0.70) and 31.9% (O/E=0.49). Observed mortality rates stratified by all risk groups did not differ between AVR and AVR/CABG (P>.08). Predictors of hospital mortality included CHF (P=.001), low cardiac output state (P=.001), prolonged ventilation (P=.0002) and previous CVA (P=.02); predictors of late mortality were coronary artery disease (P=.002), postoperative CVA (P=.01) and COPD (P=.001). STS (P=.32) and LES (P=.68) scores did not predict early or late mortality. One, 5 and 10-yr survival was respectively 95% (AVR 96%, AVR/CABG 94%), 80% (AVR 84%, AVR/CABG 77%) and 61% (AVR 63%, AVR/CABG 59 %) (P=.13).
**Conclusions:** The STS risk algorithm most closely approximates observed hospital mortality rates at different levels while LES risk algorithm often overestimated them. Neither instrument predicted early or late outcomes. In view of current surgical results and encouraging survival, octogenarians should not be deprived of surgery based on predictive risk assessment alone.

### O-20
### Cardiac FDG PET Imaging Positively Impacts Management Direction and Identifies High Risk Patients in a Multi-Center Provincial Registry (CADRE).

**Allison Hall (Supervisor: Dr. Robert S. Beanlands)**

**Background:** Better identification of individuals most likely to benefit from revascularization (revasc) could improve outcomes in pts with ischemic LV dysfunction. The PARR2 study & its post-hoc analyses showed that FDG PET-defined hibernating myocardium may be used to select such pts. Thus, a prospective provincial cardiac FDG PET registry was established in Ont. to determine the impact of FDG PET on 'real-world' decision making for revasc & if PET-defined hibernation predicts high risk pts.
**Methods/Results:** 390 PET scans were performed between 2007-2010. Inclusion criteria: A-i) LVEF = 35%; A-ii) potential candidate for revasc or heart transplant; A-iii) NYHA or CCS class II-IV symptoms; or B) lack of viability or equivocal viability findings on other non-invasive testing. Of 390 pts; 31 excluded due to sarcoidosis/aortitis (28), scan report unavailable (1), repeat scan (1) or uninterpretable scan (1). Scan interpreters indicated a recommendation where revasc/revasc workup was suggested (group(Gr)-A), revasc was not recommended (Gr-B) or recommendation was not possible without further investigation (Gr-C). The primary outcome was the combined endpoint of cardiac death, non-fatal MI & cardiac hospitalization. A perfusion/FDG mismatch score of =10% was considered significant hibernating viable myocardium. Of the 359 pts: mean age 64.4 ± 10.8 yrs, 82.7% male; LVEF =26% ± 7.7; 20.9% had renal dysfunction; 41.8% had DM. Follow up data was available for 332/359 pts (92%). Total composite event rate was 150 (45.2%). A recommendation to revascularize or not, was made in 285/332 (85.8%) pts. Of 141/285 pts (49.5%) in whom revasc was recommended, 113/141 (80.1%) were evaluated for revasc (cath) or revascularized: 99 (70.2%) ultimately revascularized. In contrast, revasc was not recommended in 144/285 pts (50.5%), among whom 32 (22.2%) underwent revasc (p<0.001 (Gr-A vs Gr-B). Pts with mismatch =10% who did not undergo revasc had more composite endpoints vs. those who were revascularized (p=0.010). When revascularization was recommended, pts who were revascularized had lower (31.0%) composite endpoint rate vs. those who were not revascularized (61.4%) p=0.002.
**Conclusions:** Recommendations based on FDG PET impacted revasc decisions. Furthermore, when revasc was performed in adherence to a recommendation to revascularize, pts had improved outcome, suggesting FDG PET results are clinically useful in selecting pts likely to benefit from revasc. In addition, the presence of hibernating myocardium, as defined by PET mismatch identifies patients who are at high risk for events if they do not undergo revascularization.

### O-21
### Three Year Clinical Outcomes Associated with the Use of the Zotarolimus-Eluting Stent in an Unrestricted Contemporary Practice

**Katie Giles (Supervisor: Dr. Christopher Glover)**

**Background:** Drug eluting stents (DES) have improved clinical outcomes by reducing the need for target vessel revascularization. However, the randomized trials from which this data originated included only patients with single, non-complex lesions and excluded patients with acute coronary syndromes. There has been a suggestion in the literature that DES may be associated with late (>1 year) stent thrombosis. The objective of this study was to evaluate clinical outcomes associated with the use of the Endeavor stent in a single center unrestricted clinical practice over a three-year period.
**Methods:** Percutaneous coronary intervention was performed at the discretion of the Interventional Cardiologist. Telephone follow-up and review of hospital and clinic records were conducted at 1 and 2 years post procedure. At 3 years, hospital and clinic records were re-reviewed and patients living outside the immediate Ottawa area were re-contacted by telephone. The primary outcome was clinically driven target vessel revascularization (TVR), target lesion revascularization (TLR) and stent thrombosis (ST) at 1, 2 and 3 years. Secondary endpoints included acute myocardial infarction (MI), cardiac death and non-cardiac death.
**Results:** Four hundred ninety four consecutive patients treated with Endeavor stents from June 2005 to Feb 2007 were followed. 717 stents were used to treat 625 lesions. Clinically-driven TLR rates were 6.2%, 1.1% and 0.3% at 1, 2 and 3 years respectively. TVR rates were 8.1%, 2.4% and 0.5% for the same time periods. The cumulative rates of TLR and TVR for the three-year period were 7.7% and 11.0%, respectively. There were 4 cases of ARC definite stent thrombosis involving 3 patients and 1 case of ARC probable stent thrombosis, all occurring within 12 months, with a rate of 0.7%. The cumulative rate of MI was 6.1% with rates at 1, 2 and 3 years of 4.0%, 1.4% and 0.6%, respectively. All cause mortality was 2.8%,

2.4% and 0.6%, whereas cardiac mortality was 1.6%, 1.8 % and 0.6% at 1, 2 and 3 year follow-up, respectively.
**Conclusions:** The rates of the clinical outcomes of TVR and TLR in this study were relatively low and were similar to those reported in earlier randomized studies, despite use of the Endeavor stent in an unrestricted population. Our results do not suggest an increased rate of late stent thrombosis.

### O-22
### A Two-Year Follow-Up of Patients Who Required Readmission to a Cardiac Surgical Intensive Care Unit

Vance Beck (Supervisor: Dr. Jim Robblee)

**Objective:** The goal of this study is to investigate outcome at two years following cardiac surgery in patients who were readmitted to the cardiac surgical intensive care unit (CSICU) and ultimately discharged alive from hospital between 2005 and 2007.
**Methods:** This study was approved by the institutional review board. The Perioperative Care Database was interrogated to identify patients who were readmitted to the CSICU following discharge to the nursing units. In this descriptive study, as part of a larger longer term follow up of this patient group, we interrogated hospital records and online databases to determine survival rates, hospital readmission, and emergency room visits. A convenience group was selected based on documented visits and admissions to the three major hospitals in Ottawa for a period of two years after cardiac surgery. Length of stay and reason for admission were also collected.
**Results:** There were 2546 cardiac procedures done during the study period, which was over a two year period from 2005 to 2007. During the study period, 2476 patients were discharged alive from the CSICU. 88 (3.6%) patients were readmitted to CSICU following discharge to the nursing unit. The mortality rate of the readmitted patients was 27%. Of the 64 patients that were eventually discharged alive from hospital, a convenience sample of 36 was investigated. 35/36 (97%) patients were alive two years after their surgery. 20/36 (56%) patients visited the emergency room with a total of 28 visits overall. The most common reasons for admission were cerebrovascular 8/28 (29%) and cardiac 6/28(21%). Twelve (33%) patients required readmission to hospital and overall this group was admitted 18 times for a total of 251 hospital days. (ALOS =13.9 days). The most common reasons for admission were gastrointestinal 9/18 (50%), respiratory 7/18 (39%), cardiac 3/18 (17%) and neurological events 3/18 (17%).
**Conclusions:** It is the conclusion of the authors that cardiac surgery patients who are readmitted to the CSICU and are subsequently discharged home are likely to survive for at least 2 years following the episode. These patients do require significant medical support following their surgery as is indicated by the frequency of emergency room visits and admissions to the hospital. Furthermore, when admission to hospital is needed, the length of stay is prolonged. The study has achieved the objective of determining that the intermediate term outcome of this very sick group of patients suggests reasonable survival. This is a question frequently asked of critical care personnel when patients are very ill following their cardiac procedure. This study is unique. There are no studies that evaluate the intermediate or long-term prognosis of patients who are readmitted to a cardiac surgical critical care unit following primary discharge to the surgical ward.

## ALLIED AND POPULATION HEALTH ORAL PRESENTATIONS

### O-23
### Addressing Nurse Fatigue at the University of Ottawa Heart Institute (UOHI)

Joshua Hambleton (Supervisor: Dr. Mirou Jaana, Telfer School of Management, University of Ottawa)

**Background:** The health of workers and risks to patients is a common concern in health care organizations where shift work is a difficult but necessary job element. Despite this, most healthcare organizations across the country, including UOHI, have not developed policies and procedures to address fatigue (CNA&RNAO, 2010). The local shift work coping mechanisms are varied between units with strategies largely founded on trial-and-error with little consideration of evidence. Encouraging active shift management is necessary to enable new nurses to adjust to shift work, middle aged nurses to continue coping with the impacts of irregular work hours, and minimize nurses leaving the organization.
**Methods:** A combination of qualitative and quantitative approaches were used for this project. First, informational interviews were conducted with nurse managers and frontline nurses (n=21) to better understand the context of fatigue management here at UOHI. These were complemented with a thorough review of the literature to evaluate local practices against recommendations. Based on this analysis, a fatigue management education session for nurses was developed. A baseline assessment of the overall level of fatigue (chronic, acute, and inter-shift), internality (locus of control: sleep, social, health, and work), personality orientation (morningness-eveningness), and satisfaction was conducted throughout the hospital (n=185). Following the establishment of the baseline measure, an educational pilot was implemented on the H5 unit (n=16) and compared against the H4 control unit (n=8). A pre-post design was used to evaluate the impact of the intervention on baseline measures.
**Results:** There is a wide discrepancy of fatigue coping strategies across the Institute, with night shift napping very prominent, yet widely varied. There are no clear guidelines on nurse napping and limited additional support for dealing with the impacts of shift work. The Intensive Care Units were found to report significantly lower fatigue scores than the upper Floors of the Institute. Surprisingly, nurses with children (<13) reported significantly lower fatigue and higher satisfaction scores. In a similar fashion, the most senior category of nurses (56-65yrs) had significantly lower fatigue scores accompanied by higher satisfaction scores. Lower fatigue was correlated to higher internality and satisfaction. During the pilot study, the reported internality of work performance dropped significantly with education. Also, the satisfaction within the control group fell significantly during the time of the study, while the fatigue increased. The trending for the education pilot was promising, but limited due to the small sample size and time constraints.

**O-24**

## Changes In Cholesterol Levels over an 18-Year Period in a Random Sample of Newfoundland Residents: Effect of Diet Versus Pharmacotherapy

**Penelope Turton (Supervisor: Dr. Marshall Godwin, Memorial University of Newfoundland)**

**Purpose:** To assess secular trends of blood cholesterol levels in a cohort of Newfoundland residents over a span of 18 years.
**Methods:** A random and representative population sample of men and women from Newfoundland were first examined in 1991-1992, and re-examined again in 2008-2009. A fasting lipid profile, including total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C), was assessed at baseline and follow-up. Respondents on lipid-lowering therapy (LLT) at baseline or for which baseline eligibility for LLT (using the 2009 Canadian lipid guidelines) could not be determined were excluded from the current analysis. A comparison of follow-up characteristics was carried out between those receiving and not receiving LLT upon re-examination. Intake of fruits and vegetables and consumption of dietary supplements (vitamins, minerals, fish oils, herbs, etc.) at follow-up were also compared between the two groups. T-tests were used to compare continuous measures and the Chi-square analysis was performed for categorical variables. The level of significance was set at 0.05.
**Results:** The study sample included 347 participants, with 47.6% comprised of men and a mean follow-up age of 63.0 yrs. Of those respondents who were eligible for LLT at baseline (n=141), 66 (46.8%) were on antilipidemic drugs when re-examined. Overall, 111 (32%) respondents were on LLT at follow-up. The mean follow-up LDL-C value for those on LLT was 2.47 mmol/L compared to 3.54 mmol/L for those not on drug therapy ($p<0.001$). Mean TC levels were also significantly lower in those on LLT (4.54 mmol/L vs. 5.66 mmol/L; $p<0.001$). Intake of fruits and vegetables was similar between both groups ($p=0.387$), with only 6-9% consuming 5 or more servings of fruits/vegetables per day. Consumption of dietary supplements also did not differ between the groups ($p=0.096$). When assessing cholesterol trends over time with no influence of antilipidemic drugs (i.e. sub-group of respondents not receiving LLT at baseline nor at follow-up; n=236), mean baseline and follow-up TC values remained unchanged (5.64 mmol/L vs. 5.66 mmol/L; $p=0.777$). There was a slight decline of LDL-C at follow-up compared to baseline (3.54 vs. 3.69 mmol/L; $p=0.030$).
**Conclusions:** According to our findings, only lipid-lowering therapy – not diet – was associated with a significant reduction in cholesterol levels. In the absence of treatment with antilipidemic drugs, we did not observe clinically relevant secular changes in lipid values over an 18-year period despite intensive promotion of "heart healthy diets."

**O-25**

## Champlain Community Heart Health Survey

**Danielle Simpson, CCPN Analyst, Minto Prevention and Rehabilitation Centre**

**Background:** Local data on Cardiovascular Disease (CVD) risk factor prevalence for the Champlain region are not available in a timely, on-going manner and are based on self-reported data. With regular monitoring of the trends in CVD and risk factor rates, real benchmarks can be established by which to measure progress and to guide future planning. The Champlain Community Heart Health Survey (CCHHS), conducted in partnership with McMaster University's Prospective Urban Rural Epidemiological (PURE) study, is a large-scale community-based survey designed to collect key information relevant to the cardiovascular health and lifestyles of the residents of the Champlain region including physical measures and laboratory data.
**Methods:** A representative random sample of postal codes in the Champlain region was selected and an information package about the CCHHS was mailed to all households. Within the selected postal codes, potential participants were screened for eligibility over the telephone. Eligible participants were mailed a participant information and consent form, food frequency questionnaire, and neighbourhood walkability questionnaire. Participants completed physical measures (blood pressure, height, weight, ECG) and laboratory tests (blood lipids, glucose) during an in-person appointment. Simple descriptive statistics were completed (ie. frequencies). Analysis took into account the clustered, weighted sampling design in order to calculate regionally representative frequencies of CVD risk factors for the Champlain District. All analyses were completed in SAS®.
**Results:** A total of 1439 participants completed the in-person assessment. Participants in the study had higher rates of secondary education and marriage than reported in the 2006 Census. The rates of diabetes (7.2%), hypertension (20.7%) and asthma (11.0%) were slightly higher in this study than in nationally reported data (see Table 1.1). Rural communities had higher rates of diabetes (10.8%), hypertension (23.8%) and angina/heart attack (5.7%) compared to the urban communities (5.7%, 19.5%, 3.0% respectively). However, urban communities had higher rates of COPD (1.8%), asthma (11.5%) and cancer (8.5%) than rural communities (1.7%, 9.8%, 5.4% respectively). For participants using medications, 42.5% of participants use blood pressure medications, 33.8% use cholesterol lowering medications and 11.3% use diabetes medications. Overall tobacco use was lower (11.3%) in this population than reported by the Census (18.0%).
**Conclusions:** The survey documented higher rates of hypertension, diabetes and asthma than previously reported by the Census. Participants living in rural communities reported lower rates of asthma, COPD and higher rates of tobacco use, diabetes, hypertension, angina/heart attack and medication use than participants from the urban communities. These results will be used to inform primary and secondary prevention efforts of cardiovascular disease in the Champlain region.

**O-26**

## Development and Psychometric Evaluation of a Theory of Planned Behaviour Physical Activity Questionnaire for Individuals at Risk for Coronary Heart Disease

**Dana Riley (Supervisor: Dr. Robert Reid)**

**Background:** Physical activity is important for the prevention of coronary heart disease (CHD), a leading cause of death in Canada. Understanding the factors that influence physical activity patterns in individuals at risk for CHD is important. The theory of planned behavior (TPB) may provide insight regarding the underlying beliefs about physical activity. Currently there are few theoretically-based measures of beliefs and intentions regarding engaging in regular

physical activity in individuals at risk for CHD. The purpose of this study was to develop an appropriate questionnaire and test its psychometric properties.
**Methods/Materials:** The current questionnaire was developed for use as part of a randomized control trial of a lifestyle intervention in individuals with a family history of CHD and =1 additional risk factor. The elicitation questionnaire was administered to a sub-sample of 16 participants, and was comprised of a series of nine open-ended questions related to behavioral, normative, and control beliefs and intentions regarding physical activity. The open-ended responses were coded and analyzed qualitatively. These responses from the open-ended questionnaire were used to develop the response options for the final version of the physical activity questionnaire. A psychometric evaluation of the questionnaire was completed in a separate sample of 10 participants over a two week period. Correlations for each scale (behavioural beliefs, normative beliefs, control beliefs and intentions) were analyzed to assess reliability and Cronbach's alpha was computed to test the internal consistency of the questionnaire.
**Results:** Qualitative analysis of the open-ended responses led to the development of a questionnaire with 18 items related to behavioural beliefs, 10 items related to normative beliefs, 9 items related to control beliefs and 6 items related to intentions. The correlation coefficients ranged from 0.100-0.909 for behavioural, 0.469-0.913 for normative, 0.108-0.826 for control and 0.179-0.975 for intentions. The internal consistency was good with Cronbach's coefficient alphas of 0.940, 0.903, 0.947 and 0.923 for behavioral belief, normative belief, control belief and intention, respectively.
**Conclusions:** The physical activity questionnaire based on the TPB was found to be a reliable measure of participants' behavioral, normative and control beliefs and intentions towards engaging in regular physical activity and it is suitable for use in individuals who are at risk of developing CHD.

### O-27
### Effects of Nordic Pole Walking on Functional Status in Patients with Moderate to Severe Heart Failure: A Randomized Controlled Trial

**Marja-Leena Keast, Physiotherapist, Minto Prevention and Rehabilitation Centre**

**Background:** Patients with heart failure are a growing population within cardiac rehabilitation programs. Usual care for these patients consists of low to moderate intensity aerobic exercise, typically walking, and upper body strength training exercise. Here we report results from the Nordic Walking Study examining the effects of 12 weeks of walking with Nordic poles versus usual care on functional status, VO2 peak, physical activity, muscle strength, body weight, waist circumference, anxiety and depressive symptoms in patients with moderate to severe heart failure.
**Methods/Materials:** Between 2008 and 2009, we conducted a randomized controlled trial at the University of Ottawa Heart Institute where 54 patients (44 men, 10 women; mean age = 62.4 ± 11.4 years) with moderate to severe heart failure (mean ejection fraction = 26.9 ± 5.0%) were randomly assigned to usual care (n=27) or Nordic pole walking (n=27) for 12 weeks. Our primary outcome was functional status assessed by distance traveled in the 6-minute walk test at 12 weeks.
**Results:** The follow-up assessment rate for our primary end point of functional status was 75%. Intention-to treat analysis of covariance indicated that Nordic pole walking improved functional status (591.3±168.8 vs. 523.7±131.9 meters traveled in 6 minutes; P=.001), waist circumference (97.8±16.5 vs. 99.8±9.7 centimeters; P=.038), right hand grip (38.1±12.3 vs. 36.2±11.7 kilograms; P=.045), left hand grip (38.4±11.2 vs. 36.5±10.9 kilograms; P=.047) and depression (3.0±2.5 vs. 4.6±3.1; P=.000) at 12 weeks compared to usual care. There were no significant differences between groups after 12 weeks for VO2 peak (6.2±2.6 vs.6.1±2.2 METs; P = .167), self reported physical activity minutes (262.5±90.9 vs. 282.1±92.5 minutes per week; P = .399), body weight (83.4±19.5 vs. 83.9±12.6 kilograms; P = .416), anxiety (4.7±2.7 vs. 4.7±3.3; P = .951).
**Conclusions:** Improvement in functional status was 13% greater in patients with moderate to severe heart failure assigned to Nordic pole walking versus usual cardiac rehabilitation care. These results need to be replicated in other studies; however, this is a promising exercise modality for this patient population.

### O-28
### Results and Applications of a Multi-Centre Chart Audit Exploring Best Practices in Heart Failure Management

**Lorraine Montoya, BSN, MAdEd, Regional Program Educator**

**Background:** As part of a larger regional quality initiative, a multi-centre chart audit exploring current practices in the management of patients with heart failure was completed. Participating centres included acute-care community hospitals within a defined local health authority. The purpose of the audit was to create a composite baseline of current practices in heart failure management.
**Methods/ Results:** After obtaining consent from each centre, files were pulled that met the definition of heart failure according to internationally recognized diagnostic codes. The audit tool was based on the Canadian Cardiovascular Outcomes Research Team (CCORT) quality indicators for measuring heart failure care in Canada. A minimum of 25 files (range: 25 – 50 files) were audited from each hospital. Patients who had died or who had been transferred were excluded. Discharge dates ranged from 2008 to early 2011. Results were presented individually to each hospital and then aggregated and analyzed to identify regional trends in the overall management of patients with heart failure. The prescription of ACE Inhibitors/ARB, provision of discharge instruction regarding medication; salt/fluid restriction, daily weights, heart failure symptoms, and physical activity instruction were identified as not meeting CCORT criteria. These are practices that have consistently shown reductions in unplanned re-admissions, in-hospital mortality, and other morbidities related to heart failure.
**Conclusions:** The results provided an overall snapshot of current practices in the management of patients with heart failure in a specific health region. They became a critical component of a knowledge translation strategy that used this objective baseline measure to provide individual performance feedback, inform tailored education strategies, and, most importantly, to provoke a variety of multi-disciplinary problem solving discussions directed towards improving care and outcomes for this complex patient population.

**O-29**

## Home Telehealth Improves HF Readmission Rates: A Case-Matched Cohort Study

Christine Struthers, RN, MScN, APN Cardiac Telehelath

**Background:** Heart Failure (HF) is the leading cause of hospitalization for patients = 65 years of age and readmission rates within 6 months can be as high as 25% to 50%. Readmission is often due to congestion and frequently related to poor self-care management. Patients referred to a telehome monitoring (THM) program are provided with a home monitor and scale to transmit their daily weight and vital signs by regular phone line to a central station manned by a nurse. The purpose of this study is to examine the impact of a THM program on the 6 month HF readmission rates.
**Methods/Results:** A case control study of heart failure (HF) patients referred to a home teleheath program in 2007 & 2008 was undertaken to compare readmission rates with a control group identified from the CIHI Discharge Abstract Database of a quaternary center. HF patients were matched by age (+/-3yrs), sex, & EF range (<30, 30-39, 40-50, >50) producing 91 pairs. Exclusion criteria included no documented EF, patients admitted for ventricular assist device &/or heart transplant (HTX) surgeries, elective admission for HTX assessment & loss to follow-up. Data was analyzed using a repeated measures design comparing the pre versus post 6 month readmission rates between the 2 cohorts. The average age was 70 years (range 30-90), 23% were female, and the majority of patients had an EF of <30% (63.7 %). Results for the other EF ranges included EF 30-39 (13.2%), EF 40-50 (8%), and EF>50 (14.3%). The pre 6 month readmission rate (= 1) for the THM group was 56% compared to the post 6 month readmission rate of 9.9%. In comparison the control group had a 19.8% = 1 pre 6 month readmission rate compared to 41.8 % 6 months post admission. Limitations of the study may be related to the use of 3 main matching variables. Capturing onset of HF, medication regimen and precipitating causes of HF admission may contribute to further studies.
**Conclusions:** There was a significant difference in the pre and post 6 month readmission rates (= 1) between the 2 cohorts (P<0.001). Further research is required to determine positive patient outcomes related to follow-up using home telehealth technologies.

**O-30**

## Using IVR to Improve Disease Management and Compliance with ACS Best Practice Guidelines

Christine Struthers, RN, MScN, APN Cardiac Telehelath

**Background:** Acute coronary syndrome (ACS) is a significant public-health problem in Canada and worldwide with 20,926 Canadians dying of an acute myocardial infarction and 42,619 dying of ischemic heart disease in 1999. Large clinical trials have provided evidence for the development of standardized best practice guidelines (BPG) and compliance with these guidelines have significantly improved survival. Despite the development and dissemination of BPG, their application in patients with acute coronary syndrome (ACS) is suboptimal. The purpose of this study is to evaluate whether the use of an Interactive Voice Response (IVR) system for patient follow-up improves compliance with ACS BPGs.
**Methods/Results:** A total of 1608 ACS (MI, STEMI or NSTEMI, Unstable Angina) patients from a quaternary center participated in this study in 2006-2009. A randomized control trial design was used with 2 groups: IVR (n=803) & Usual Care (UC n=805). Inclusion criteria included English or French speaking adults (=18 years) discharged with an ACS and telephone service. Patients discharged to a care facility were excluded. The IVR group received 5 automated calls at 1, 3, 6, 9, and 12 months consisting of predetermined questions related to medication management, smoking cessation, diet, exercise and education as recommended by the ACC/AHA BPG. Responses were captured in a database allowing for interventions to maintain patients on BPG as needed. Patient satisfaction was measured by survey at 1 year. The analysis was conducted on an intention to treat basis. The primary composite outcome of increased compliance with medication and decreased adverse events (ER visits, hospitalization, and unplanned visit) and discreet secondary outcomes such as ER visits were analyzed by logistic regression model. The analysis of patient satisfaction was presented by frequencies and percentage.There was no significant baseline characteristics difference between the IVR and UC groups. The primary composite outcome was significantly different between the IVR & UC group (p<0.001). The discreet secondary outcome of compliance with medications was statistically significant for the IVR group (p<0.001). There were no significant statistical differences between the 2 groups for the discreet secondary outcomes of ER and unplanned visits and hospitalization. Patient satisfaction in the IVR group was high with 85% willing to use the system again and 90 % reporting that using IVR was a good follow-up method.
**Conclusions:** Patients followed by IVR are more likely to have increased compliance with medication and decreased adverse events compared to patients using usual care. Using IVR is an easy and cost effective way of following a large number of patients to ensure compliance with BPG producing positive outcomes.

**O-31**

## Cost Analysis of the Ottawa Model for Smoking Cessation as Implemented at an Ontario Tertiary Care Cardiac Hospital

Kerri-Anne Mullen (Supervisor: Dr. Robert Reid)

**Introduction:** From the perspective of the hospital, the objective of this study was to estimate the cost savings realized by a systematic smoking cessation intervention provided to patients admitted to a tertiary care cardiac institution. The clinical intervention being examined was the Ottawa Model for Smoking Cessation (OMSC), that involves systematically identifying, treating (with bedside counseling and pharmacotherapy), and offering follow-up support to smokers for up to 6 months.
**Methods:** A costing model was developed in order to estimate both the costs of operating the program and the savings resulting from reduced hospitalizations. Resource-use data was derived from actual OMSC operating costs and the costs of hospitalization for specific case mix groups were derived from the Ontario Case Costing Initiative. To derive estimates of the potential benefits of the OMSC intervention on re-hospitalization, data from a previous study by Mohiuddin (2007) was used.
**Results:** In 2008-2009, UOHI treated 1491 smokers with CHD using the OMSC protocol. The projected continuous abstinence rate at 24 months was 30.0% in the intervention group and 23.5% in the usual care group. The intervention was estimated to prevent 35 hospitalizations due to myocardial infarction, 27 hospitalizations due

to unstable angina, 4 hospitalizations due to arrhythmias, and 9 hospitalizations due to heart failure. The total operating cost of the OMSC program was $201,105. The net potential cost savings of the program can be estimated by subtracting the costs of the program ($201,105) from the potential cost savings due to reduced hospitalizations ($714,000) arriving at a value of $512,895. Return on investment was 355% (calculated by dividing program savings into program costs) and the total number of hospital bed-days prevented was estimated at 476 days.
**Conclusions:** Provision of the OMSC to patients admitted to hospital with CHD may lead to cost savings due to a reduction in cardiovascular re-hospitalization.

### O-32
### Effectiveness of the Ottawa Model for Smoking Cessation in Cardiac Care Units Across Canada
**Kerri-Anne Mullen (Supervisor: Dr. Robert Reid)**

**Background:** Cigarette smoking is a primary modifiable risk factor in the development of cardiovascular diseases (CVD). Unfortunately, most hospitals in Canada are still without systems and protocols to adequately identify and treat admitted tobacco-users. The Ottawa Model for Smoking Cessation (OMSC) is an effective clinical smoking cessation intervention that has been implemented in nearly 70 hospitals across Canada. Among cardiac care units in Canada, the objectives of this investigation were to assess: 1) the prevalence and characteristics of admitted smokers; 2) baseline smoking cessation practices; and 3) the effectiveness of OMSC implementation.
**Methods/Results:** A before-and-after study was completed at 12 Canadian hospitals implementing the OMSC with units specializing in cardiac care. The evaluation was guided by the RE-AIM (reach, efficacy, adoption, and implementation) framework. Reach was defined as the proportion of expected cardiac smokers identified and offered treatment. Efficacy was measured as the change in 6-month smoking cessation rates following OMSC implementation. Adoption was the proportion of possible cardiac units in each hospital that implemented the intervention. Implementation pertained to the number of recommended best practices for clinical smoking cessation put in place. Average smoking prevalence was 22.4% among a consecutive series of cardiac admissions screened during pre-implementation data collection. Throughout a one-year observation period following implementation, 3403 smokers admitted to participating units received the OMSC intervention, representing 56.0% of expected cardiac smokers. OMSC efficacy was measured in the first 5 hospitals (16 units) to complete the before-and-after implementation assessments (n=1094). Controlling for hospital, the 6-month smoking cessation rate increased by 12.7% after introducing the OMSC (from 20.6% to 33.3%; OR = 1.90; CI = 1.41 – 2.54; Z = 4.27; I 2 = 0%; p <0.001). Overall, 69.2% of all cardiac units in participating hospitals adopted the program (36 of 52) and, on average, 5/10 best practices for clinical smoking cessation were in place at baseline, compared to 9/10 following the one-year observation period.
**Conclusions:** Our study demonstrates that, in general, Canadian hospitals specializing in cardiac care appear to do a satisfactory job identifying smoking status of admitted patients; however, most do not provide sufficient treatment to support continued smoking abstinence. Implementation of the OMSC significantly improves long-term smoking cessation rates and can be easily incorporated into hospital routines. Annually, an estimated 1.5 million hospital-days are associated with tobacco-use in Canada - the majority due to CVD. Considering its impact on CVD-related mortality, morbidity, and re-hospitalization, smoking cessation is a priority for cardiac patients.

### O-33
### Perceptions of Barriers and Facilitators to Smoking Cessation Among Pregnant Smokers in the Baffin Region of Nunavut
**Chantal Nelson (Supervisor: Dr. Robert Reid)**

**Objectives:** Recent statistics show that approximately 80% of pregnant women smoke in Nunavut, which is much higher than the national average of 18.5%. The reasons underlying women's smoking patterns are complex, which reflect multiple and interacting social, cultural, economic, and biological influences. Currently, there is no research done among pregnant women in Nunavut to explain this regional variation, therefore the objective of this study was to build a knowledge base for understanding smoking behaviours among pregnant women in Nunavut and identify and characterize the perceptions of barriers and facilitators to smoking cessation among pregnant Inuit women.
**Methods/Results:** A community-based study was conducted in Iqaluit, Nunavut, between May-June 2010 using semi-structured interviews (n=17) with pregnant Inuit women who were currently smoking, or had smoked at least 1 cigarette since they learned they were pregnant. Approximately half of the women interviewed (n=8) stated they started smoking more when they found out they were pregnant (current pregnancy) than in pre-pregnancy. All women interviewed who were flown in to Iqaluit for the delivery of their babies stated that they increased cigarette smoking since leaving their communities, some as much as 2-3 times their regular daily cigarette consumption. When asked about the potential risks to the baby, most women identified that they understood there were health risks to their fetus, but this was not sufficient motivation to have them quit. Women who have had previous pregnancies, had children who manifested symptoms related to tobacco exposure, were still smoking during their current pregnancy. When asked if the women knew where they could go for help or support to quit smoking, the majority stated that they did not know of any existing programs or did not know where to go. All women reported stress as the main reason for their continued smoking throughout their pregnancy. Stress factors included financial situations, housing instability, troubled partner relationships and single parenthood. All women identified that smoking was used as a coping mechanism as it helped them relax, and temporarily relieved their anxiety. The women were asked what they feel could help them quit smoking, three main ideas emerged: nicotine replacement therapy, someone to talk to and community groups/activities.
**Conclusions:** This study presents an exploratory analysis of the smoking behaviours of Inuit women in Iqaluit, Nunavut. Women in the study revealed that their smoking increased during pregnancy, particularly when arriving in Iqaluit just prior to their expected due date. Stress, boredom and the social experience of smoking emerged as the biggest themes of the underlying reasons why women continue to smoke during their pregnancy. Smoking cessation poses a major challenge, primarily as a pressing public health problem. Few studies have focused on the smoking behaviours of pregnant Inuit women in Nunavut. This is one of the first studies detailing

the women's perceptions on their smoking behaviours during their pregnancy. Further research is required to address smoking among higher-risk pregnancies.

## FUNDAMENTAL SCIENCE POSTER PRESENTATIONS

### P-1
### Identification of Nuclear Localization Signal in IRF2BP2

**Allen Teng (Supervisor: Dr. Alexandre Stewart)**

Interferon regulatory factor 2 binding protein 2 (IRF2BP2) is an ischemia-inducible and muscle-enriched transcription factor required to activate vascular endothelial growth factor-A (VEGF-A) expression in muscles. Endogenous IRF2BP2 is found in the nuclei of cardiac and skeletal muscle cells under normoxic condition, but is largely cytoplasmic in ischemic tissues. The mechanism that controls nucleocytoplasmic localization of IRF2BP2 is not yet known. Here, we mapped the nuclear localization signal (NLS) to an evolutionarily conserved 354ARKRKSP361 sequence in IRF2BP2 with the use of green fluorescent protein recombinant proteins. Whereas the arginine and lysine were necessary for nuclear localization, they were not sufficient. Nuclear targeting required the phosphorylation of serine 360 (S360). Alanine substitution at this site abolished IRF2BP2 nuclear entry. Thus, loss of protein kinase activity in ischemic tissues likely accounts for cytoplasmic accumulation of IRF2BP2 and impaired revascularization.

### P-2
### Culture of Circulating Progenitor Cells on a 3D Collagen Matrix Enhances Their Therapeutic Effect in a Model of Hindlimb Ischemia

**Chenchen Hou (Supervisor: Dr. Erik J Suuronen)**

**Introduction:** Transplantation of circulating progenitor cells (CPCs) in cell therapy has the potential to restore function to ischemic tissue, mediated by neovascularization. Herein, we present novel findings that demonstrate how the therapeutic potential of CPCs may be enhanced by pre-conditioning in a 3D culture system.
**Methods/Results:** Collagen matrix was prepared by cross-linking collagen with chondroitin sulfate-C using glutaraldehyde. CPCs were isolated from human donors, and cultured on a 3D matrix or on fibronectin as control. Flow cytometry was performed for CPC phenotype analysis. Hindlimb ischemia was induced in nude mice, followed by transplantation of CPCs (raised on fibronectin or matrix), or PBS as a control. Limb perfusion was assessed over time using laser Doppler. On day 14, serum was collected for cytokine analysis to investigate the host paracrine response triggered by treatment. Compared to culture on fibronectin, CPCs on collagen matrix were enriched in the number of CPCs expressing hematopoietic stem cell markers CD133 and CD34 (by 4.2- and 2.6-fold, respectively) and endothelial markers CD144 and CD31 (by 3.2- and 2.3-fold, respectively), $P<0.05$ for all. After PBS treatment, hindlimb perfusion was further reduced by 29% at day 4 ($P=0.05$), whereas matrix-cultured cell-injected mice showed a 51% increase that was sustained until day 14, and constituted a 42% increase vs. fibronectin ($P=0.02$). Circulating pro-angiogenic cytokines SDF-1 (2.6-fold; $P=0.03$), G-CSF (2.9-fold; $P=0.02$), VEGF (1.8-fold; $P=0.04$) and SCF (1.8-fold; $P=0.03$) were elevated, in the matrix-cultured CPC group compared to PBS.
**Conclusion:** The expansion of CPCs on collagen matrices is more effective for generating therapeutically relevant cell populations. Ultimately, matrix-culture may offer a novel strategy to improve CPC therapy for ischemic diseases.

### P-3
### Synthesis and Characterization of Chitosan-Derived Microgels for Cardiovascular Tissue Engineering

**Donna Padavan (Supervisor: Dr. Erik J Suuronen)**

**Background:** Cell-based therapies for the treatment of CAD, including the use of circulating progenitor cells (CPCs), can promote neovascularization and cardiac function. However, cell therapy is hindered by a low rate of engraftment and low persistence of cells in the target tissue. Tissue engineering offers the possibility of using biomaterials, to improve the retention, survival, and function of transplanted and/or recruited cells. Thus, our objective is to develop and characterize new materials (microgels), which can support transplanted cells.
**Methods/Results:** A chitosan derivative, N-[(2-hydroxy-3-trimethylammonium)propyl] chitosan chloride (HTCC), was synthesized by mixing glycidyltrimethylammonium chloride (GTMAC) and chitosan in deionized water for 24h at 85°C. Two material scaffolds were developed: (a) HTCC was ionically cross-linked by sodium tripolyphosphate; and (b) low, medium and high molecular weights of pure chitosan microgels were obtained via ionic cross-linking with beta-glycerophosphate disodium salt in HCl aqueous solutions. Preliminary biocompatibility assessments of these microgels were conducted using CPCs. Cells were incubated for 1 and 7 days and viability was evaluated using the WST-1 assay. Successful synthesis of HTCC was highly dependent on the 4:1 molar ratio of GTMAC to amino groups of chitosan. The presence of quaternary amino groups on chitosan was confirmed by characteristic absorption bands identified by Fourier Transform Infrared (FTIR) spectrometer analysis, and also by its ability to solubilize in water at neutral pH. Following ionic cross-linking, both microgels were initially aqueous, and as temperature increased, their viscosity increased leading to semi-transparent microgels. Microgels did not revert back to an aqueous state as the temperature decreased. CPCs seeded onto microgel-coated 96- and 24-well cell culture plates, and compared to CPCs grown on fibronectin, helped to establish structure-toxicity relationships. After day 1, CPCs cultured on microgel-coated wells appeared clustered and rounded with no evidence of cytoplasmic extension; whereas, CPCs cultured on fibronectin adhered, spread, and cellular processes were observed. By day 7, CPCs cultured on microgels were viable; however, cell numbers were relatively low.
**Conclusions:** The developed chitosan-derivative microgels are natural polymer-based, physically cross-linked materials having improved aqueous solubility that are synthesized without the use of chemical cross-linkers. The presence of functional groups in the chitosan polymer chains enhances its versatility. Preliminary results indicate that the microgels are not toxic to CPCs. The low cell number may be corrected by varying the cross-linker concentration. These microgels are attractive for tissue engineering applications because of their physical properties and biological parameters.

**P-4**

## Quantitative Reconstruction of Small Animal Multiplexed Multi-Pinhole SPECT with Scatter and Attenuation Correction

Jared Strydhorst (Supervisor: Dr. Glenn Wells)

**Background:** Small animal SPECT scanners capable of high resolution nuclear imaging of small animals are available, but quantitative imaging is still a challenge. The detected photons are affected by attenuation in the body of the subject and scatter from both the subject and the collimator. Attenuation in small animal SPECT has previously been measured by Hwang et al. In the same paper they also simulate, using Monte Carlo methods, the scatter from a small animal. In clinical SPECT the dominant source of scatter is the subject itself. In previous work, we've measured the total scatter in multiplexed multi-pinhole SPECT empirically, and estimated the total collimator scatter to be about 5% and subject scatter for a rat sized subject to also be about 5%, for a total scatter contribution of about 10%. In this work we investigate development of full scatter and CT-based attenuation compensation to enable accurate quantitative multiplexed multi-pinhole SPECT of small animals.

**Phantom:** We constructed a phantom, consisting of three concentric chambers. The central chamber, with a diameter of 10 mm was filled with water. The next chamber, with a diameter of 25 mm, was filled with water containing 853 µCi (31.6 kBq) of 99m Tc for this experiment. As the aim of this work was to demonstrate quantitatively accurate reconstruction with no dependence on the size of the subject, filling the outer chamber (diameter: 42 mm) with water or leaving it empty permitted the same measurements to be done and compared with both a rat-sized and mouse-sized phantom.

**Scans:** The phantom was scanned with the nanoSPECT small animal SPECT scanner (Bioscan, Washington, DC) with the APT2 apertures comprising 9 multiplexed pinholes 2.0 mm in diameter. A total of 48 projections were acquired, with an acquisition time of 180s per projection. The scan was repeated with the outer chamber empty and with water. Projection data was recorded for two energy windows, one centered at the photopeak (126 – 154 keV) and a second window below the photopeak centered at 110 keV (96.5 – 124.5 keV).

To provide a reference for absolute calibration, a point source with a known activity of 119 µCi (4403 kBq) was also scanned.

A CT scan of the phantom was also acquired with a tube voltage of 45 kVp. The CT was reconstructed by filtered backprojection (FBP) at a resolution of 0.2 mm, with beam hardening correction.

**Reconstruction:** The attenuation map was created by downsampling the reconstructed CT to a resolution of 0.6 mm and scaling the density to obtain a linear attenuation coefficient of 0.15 cm-1 (linear attenuation of 150 keV photons in water, ref. NIST). From the attenuation map, the cumulative photon attenuation for each voxel at each projection was calculated by ray-tracing through the attenuation map. Both the point source and the phantoms were reconstructed using an OSEM algorithm, run for 6 iterations and 6 subsets per iteration.

Attenuation correction was done during OSEM by scaling the current estimate by the cumulative attenuation map for each projection prior to the forward projection step and similarly scaling the backprojected ratio when updating the estimated source distribution.Scatter compensation was performed using the dual energy window method to 'pre-correct' the projection data; the scatter in each pixel of the projection was estimated from the activity measured in the lower energy window. The total scatter in the photopeak window is assumed to be approximately half of the scatter measured in the downscatter window, though this is a heuristic value based on Jaszczak, et al.

**Results:** The point source had a measured activity of 4403 kBq. The total of the nominal activity in the reconstruction of the point source was 176955, resulting in a conversion factor of 0.0249 kBq/count.

The total initial activity concentration in the hot region of the phantom was 1435 kBq/mL. All measurements were corrected for decay for comparison with the initial concentration. Attenuation correction partially corrects for the activity lost to attenuation, resulting in a calculated activity concentration within 10% of the true concentration. With the outer chamber empty, attenuation correction increases the measured activity by 20%, with the chamber full attenuation correction increases the measured activity by 37%. In the (cold) centre chamber the effects of attenuation correction were even more noticeable, where AC increased the apparent activity by 36% and 48% with the outer chamber empty and full, respectively.

The effect of scatter correction was the same with the outer chamber full and with it empty. Scatter correction reduced the measured activity by 6-7% in the hot region and by 17-18% in the central cold region. Work still remains to be done on the system matrix model to improve the system matrix model, which may improve reconstruction of cold regions.

**P-5**

## A Collagen Matrix Activates the ERK Pathway and Improves the Survival and Function of Endothelial Progenitor Cells

Jenelle Marier (Supervisor: Dr. Erik J Suuronen)

**Introduction:** Biomaterials are being developed to augment the efficacy of endothelial progenitor cell (EPC) therapy. EPC transplantation with a collagen matrix was previously shown to be superior to EPCs alone for restoring function to ischemic tissue. This study explored a possible mechanism through which the matrix may confer improved EPC therapy, specifically investigating activation of the ERK pathway, which is involved in the transduction of external signals to normalize intracellular activities.

**Methods/Results:** Human EPCs were cultured on fibronectin (control) or a collagen/chondroitin sulfate-C matrix, cross-linked with glutaraldehyde. Cell lysates were probed for ERK using Western blotting. Flow cytometry was performed to assess cultures for progenitor cells (CD34, CD133), for endothelial cells (CD31, CD144); and for proliferation (EdU). Migration and adhesion of cells, with or without ERK inhibitor (PD98059), were assessed. Finally, cells were exposed to serum deprivation, and viability was assessed using 7-AAD staining. Increased ERK1 (1.4-fold) and ERK2 (1.1-fold) phosphorylation was observed in matrix-cultured cells ($p=0.05$), indicative of greater ERK activity. Proliferation of CD133+ and CD133+CD34+ cells was increased on the matrix compared to fibronectin (by 2.9- and 1.6-fold, respectively; $p=0.02$). Adhesion potential was greater on collagen (4.0-fold; $p=0.02$), and 40% ($p=0.02$) more matrix-cultured cells were observed to migrate. When ERK inhibitor was applied, the differences between treatments in adhesion and migration were

abrogated. After serum deprivation, there were 3.8-fold (p=0.07) more viable CD34+ cells and 7.8-fold (p=0.02) more viable CD133+ cells on collagen matrix.
**Conclusions:** A collagen matrix confers pro-survival and proliferative signals for progenitor cells, and enhances cell adhesion and migration capacity, mediated by the up-regulation of ERK. The use of collagen matrices is promising for enhancing cell-based regenerative therapies.

**P-6**
**Circulating Progenitor Cell Viability and Collagen Hydrogel Properties are Dependent on the Interactions between the Cells and the Biomaterial**
**Kimberly McEwan (Supervisor: Dr. Erik J Suuronen)**

**Background:** Injectable hydrogels are attractive for biomedical applications to deliver therapeutic cells in a minimally invasive manner. While the materials' properties are often characterized, the effects that the cells and materials have on one another is often overlooked. This study investigated the interactions between collagen-based hydrogels and different additives (cross-linkers, cells and microspheres).
**Methods/Results:** Type-I collagen (1%) was cross-linked with a 1:1 molar ratio of EDC/NHS (6.5, 13, and 26mM). Human circulating progenitor cells (CPCs) were incorporated into hydrogels either in PBS or glycine prior to thermogelation (cell densities of 2.5×105, 5.0×105, and 1.0×106 per 500µL of matrix were tested). A live/dead staining kit was used to assess 24-hour CPC viability. Rheological properties of matrices with varying cross-linker concentrations, cell densities and with alginate microspheres were measured using a Brookfield R/S-Plus Rheometer. The EDC/NHS cross-linker induced cell death in a dose-dependent manner, and cell density had no effect in preventing this cell death. However, the addition of cells to the matrix mixture in glycine did improve CPC viability compared to addition of cells in PBS (74.4% versus 44.9%, respectively; p=0.03), and did not differ from baseline (91.8%; p=0.2). Doubling the EDC/NHS concentration resulted in a 0.7-fold reduction in gelation time (p=0.004), while halving it resulted in a 1.8-fold increase (p<0.0001). Maximum viscosity increased with increasing EDC/NHS concentration. The addition of 1.0×106 cells reduced matrix gelation time by 22.6% and 18.2% compared to the matrix with no cells (p=0.001) and 2.5×105 cells (p=0.01). Maximum viscosity reached for matrices with cells was significantly greater than matrices without cells (p=0.03). The addition of microspheres to the matrix reduced gelation time by 10% (p=0.03) and increase maximum viscosity by 23% (p=0.02).
**Conclusions:** Cell-material and microsphere-material interactions should be taken into consideration in the development of delivery scaffolds. These interactions can affect both the physical characteristics of the material and the function of the cellular components. A better understanding of how materials and cells (and other additives) respond to each other will help towards the goal of improving scaffolds for regenerative therapy.

**P-7**
**Durability and Immunological Correlates with Implanted Porcine Heart Valves**
**Noor Al-Attar (Supervisor: Dr. Marc Ruel)**

**Background:** Studies have shown that porcine tissue expresses antigens similar to those expressed on human erythrocytes defined by the ABO phenotype convention. Pig erythrocytes, however, have an AO system of blood phenotyping dictated by A and H antigens, respectively.We aimed to identify an interaction between porcine blood groups and human recipients after bioprosthetic aortic valve replacements. We hypothesized that dynamic interplay between human antibodies and the newly inserted valve antigens may influence the structural integrity, function and durability of the porcine valve.
**Methods and Results:**The project is composed of 3 phases of experimental procedures. The first experimental phase involves the development of a method to extract and genotype DNA from freshly harvested porcine aortic valves. The second experimental phase utilizes the developed DNA extraction method on both fixed non-xenograft porcine valves and sample xenografts. As with the first experimental phase, the molecular characterization is accomplished by way of PCR-amplification of the ABO blood group gene expressing for the glycosyltransferases. Lastly, the final experimental phase focuses on genotyping a select group of bioprostheses, in particular those that have lasted 16 or more years after the initial aortic valve replacement surgery. Bioprosthesis genotype data (representing porcine blood type) will be stratified based on longevity and correlated with respective human recipient blood type (retrieved from patient database). Confirmation of porcine genotypes will be done by way of sequencing amplified PCR products and alignment to NCBI porcine sequence.Thus far we have been able to devise a DNA extraction protocol that implements a flash freeze method with liquid nitrogen. This is followed by high sonication to aid in tissue breakdown and cell lysis, mixed with phenol:chloroform:isopropanol washes that aid in DNA extraction and purification. The protocol was successful in extracting DNA from both fresh porcine aortic valves and xenografts. Using custom porcine-specific primers on these DNA samples, we were able to amplify the regions of the ABO blood group gene specific to blood type alleles A and O. Upcoming actions include the sequencing of the amplified products to verify the homology level against the intended sequences, reflecting a good-yield DNA protocol and high efficiency of the designed primers.
**Conclusions:** Initial experimentation has demonstrated our ability to extract and characterize DNA from both fresh and fixed porcine tissue. With completion of our third phase of experimentation, we predict that the durability of a given implanted bioprosthesis may be impacted by its tissue blood type and that of its human recipient.

**P-8**
**Aortic Cusp Prolapse Repair: Effect of SURGICAL Technique on Leaflet Dynamics and Stress**
**Stefano Mastrobuoni (Supervisor: Dr. Munir Boodhwani)**

**Objective:** Cusp prolapse correction is frequently required during aortic valve sparing and repair surgery. While a number of surgical techniques have been proposed to correct cusp prolapse, their effects on leaflet dynamics and stress remain unknown. We compared three surgical techniques for varying degrees of cusp prolapse correction and examined their effects on leaflet dynamics and stress.

**Methods:** Preoperative data from 3D echocardiography in patients with aortic dilatation and aortic insufficiency (AI) were combined with in-vitro experimental data, to create computational models of the aortic valve using dynamic finite element analysis. In this model, correction of aortic dilation resulted in residual AI due to single cusp prolapse. The prolapsing cusp was repaired within the model using central free margin plication, commissural plication or free margin resuspension. Outcome measures included valve opening/closing characteristics, residual AI and coaptation surface, and mechanical leaflet stress.
**Results:** All three methods of prolapse correction yielded a competent valve when the cusp free margin was shortened to exclude the excess free margin length. Correction greater than the excess free margin length by 1 to 2 mm led to cusp restriction, a 10 – 20% reduction in coaptation surface and orifice area, and residual AI. Leaflet stresses were upto 3-fold higher than normal with commissural plication and lowest with the free margin resuspension technique ($p < 0.001$). Free margin resuspension also resulted in valve opening and closing most similar to healthy valves, whereas plication techniques produced slower valve closing.
**Conclusions:** While all techniques for cusp prolapse correction can yield a competent valve, free margin resuspension is most effective at restoring physiologic valve function and minimizes leaflet stress compared to central or commissural free margin plication. These properties may reduce cusp calcification and tears, improving long-term durability following cusp repair.

**P-9**

### ROCK1/2 Regulation as a Marker of Cardiac Hypertrophy

**Steven Moreau (Supervisor: Dr. Pasan Fernando and Dr. Jean DaSilva)**

Bac**kground:** Cardiac hypertrophy is a compensatory mechanism in direct response to elevated pressure overload on the heart. During cardiac hypertrophy, cardiomyocyte sacromeric reorganization leads to an increase in overall heart size. Cell signalling factors involved in this process include members of the RhoA and ROCK1/2 pathway. These factors can be activated in vitro through the ß-adrenergic receptor agonist isoproterenol (ISO). Hydroxyfasudil is an inhibitor of active ROCK1/2 and may allow assessment of its activity during cardiac hypertrophy. Here we began to explore the utility of N-[11C]-methyl-hydroxyfasudil in vitro as a potential PET tracer for imaging cardiac hypertrophy in vivo.
**Methods/Results:** Primary cardiomyocytes isolated from neonatal rat pups as well as H9C2 rat ventricular cell line were dosed with ISO from 2-50 µM over several timepoints. Cell and nuclear size were examined using H&E and DAPI respectively. The extent of hypertrophy was examined over 72h in ISO versus vehicle treated cells. To examine hypertrophy at the molecular level, fluorescent cell staining was used for detection of mTOR and ERK1/2 as well as their activated states. Following these studies, ROCK1/2 activity will be examined by cell staining and activity assays in ISO treated cells. N-[11C]-methyl-hydroxyfasudil binding under hypertrophic and control conditions will be evaluated and correlated to ROCK1/2 activity.
H9C2 cells showed a 1.5-1.8 fold increase in nuclear size after treatment with 10µM ISO over 72h. H&E staining showed an increase in overall cell size after similar ISO treatment of approximately twice the control cell size. Cell viability remained above 90% during the ISO treatment.
**Conclusions:** This data together with the molecular analysis of ROCK1/2 signalling will help us derive a correlation between N-[11C]-methyl-hydroxyfasudil binding and cell hypertrophy in vitro. These results may demonstrate the use of N-[11C]-methyl-hydroxyfasudil to detect cardiac hypertrophy in vivo.

**P-10**

### Effects of Cryopreservation on Peripheral Blood Mononuclear Cells and Circulating Angiogenic Cells

**Tanja Sofrenovic (Supervisor: Dr. Erik J Suuronen)**

**Introduction:** Regenerative medicine has become an appealing therapeutic method; however, stem and progenitor cells are not always freshly available. Cryopreservation offers a way to freeze the cells as they are generated, for storage and transport until required for cell therapy. This process preserves cells by dramatically reducing biological metabolism at low temperatures. Nevertheless, the effects of cryopreservation on the phenotype and function of the cells, in this case peripheral blood mononuclear cells (PBMCs) and the culture-generated circulating angiogenic cells (CACs) shown to be involved in neovascularization, have not been extensively studied.
**Methods:** PBMCs were extracted from healthy donors (n=7) using density gradient centrifugation. The freshly isolated cells were either analyzed or frozen with liquid nitrogen in media containing 6% plasma serum and 5% dimethyl sulfoxide. After being frozen for 1 day or 28 days, the PBMCs were thawed and analyzed or cultured on fibronectin coated plated with endothelial basal media for 4 days to generate CACs. Analysis of the cells consisted of flow cytometry, for viability and surface markers: CD31, an endothelial cell marker; L-selectin, an adhesion factor; CD34, and KDR (VEGFR2), markers of stem and progenitor cells. Functional analysis was also conducted to assess low-density-lipoprotein (LDL) uptake and lectin binding, as well as the adhesion and migration potential.
**Results:** The viability of PBMCs and CACs was not significantly affected by cryopreservation. In PBMCs: CD34 and VEGFR2 expression increased both at day 1 and day 28 thaws ($p<0.05$), whereas the adhesion marker L-selectin was decreased ($p<0.05$), and endothelial marker CD31 remained unchanged. There was no significant difference in expression of CD31, CD34, VEGFR2 and L-selectin markers in CACs derived from cryopreserved PBMC samples. Uptake of LDL and lectin binding, properties associated with the more therapeutic CACs, as well as adhesion and migration capabilities of PBMCs and CACs were not significantly affected after cryopreservation.
C**onclusions:** Cryopreservation of PBMCs did not affect the cells' viability, nor their migrative and adhesive functions. PBMCs were affected phenotypically, with changes in CD34, VEGFR2 and L-selectin expression. CAC phenotype and function were not affected by cryopreservation. Overall, it appears that the more therapeutic CACs tolerate cryopreservation better than the heterogeneous PBMC population.

**P-11**

### Mitochondrial Fusion is Actively Repressed in Primary Tissues, Requires ROS and is Activated Specifically in Response to Stress

**Timothy Shutt (Supervisor: Dr. Heidi McBride)**

**Background:** Mitochondria are dynamic double-membrane-bound organelles that are capable of both fission and fusion. It is thought that the dynamics between the relative amounts of ongoing fission or fusion lead to the spectrum of mitochondrial morphologies that are observed within any particular cell, ranging from a punctate appearance (more fission) to a reticulated network (more fusion). Changes in mitochondrial morphology may reflect mitochondrial function and are associated with several factors including cell cycle, stress, and apoptosis. However, the extent of mitochondrial dynamics in primary tissues has not been established with confidence since there has not been any assay system to quantify these processes in vivo or in vitro.
**Methods/Results:** Using a bimolecular complementation approach that follows the re-assembly of luciferase upon the mixing of mitochondria in vitro, we have developed a robust assay to quantify mitochondrial fusion independently of fission. Using this approach we show first that mitochondrial fusion is stimulated by cytosols derived from cultured, transformed cells, but is inhibited by cytosols isolated from primary tissues. In addition, we demonstrate that increased levels of reactive oxygen species (ROS) are able to stimulate mitochondrial fusion. Meanwhile, the presence of antioxidants completely inhibits fusion. The inhibition of mitochondrial fusion by antioxidants is mediated, at least in part, by regulation of the stability of Opa1, a known factor of mitochondrial inner-membrane fusion.
**Conclusions:** These data indicate that mitochondrial fusion may be actively repressed in primary tissue. However, upon stress or insult conditions that generate ROS, mitochondrial fusion is stimulated. Importantly, our data also show that mitochondrial fusion absolutely requires ROS, which may ensure that only actively respiring mitochondria are able to fuse and share their contents with neighbouring organelles. Therefore our results suggest a model that mitochondrial fusion may not occur in primary cells unless there is a localized stress response. The mechanistic difference between the fusion potential of cytosols derived from primary cells like cardiomyocytes compared to cultured, transformed cells will be the subject of future work.

**P-12**

## Functional Relationship of the COL4A1/COL4A2 Locus on Chromosome 13q34 to Coronary Artery Disease (CAD)

**Adam Turner (Supervisor: Dr. Ruth McPherson)**

Type IV collagen triple helices constitute the major structural component of basement membranes, consisting primarily of 2 COL4A1 chains arranged with 1 COL4A2 chain. COL4A1 and COL4A2 also have important functional roles in angiogenesis, and mutations are associated with diverse vascular abnormalities. The COL4A1 and COL4A2 genes on chromosome 13 are arranged in a head-to-head conformation, and uniquely share a common, bidirectional promoter. In the Ottawa Heart Study (OHS) and as part of CARDIoGRAM, a large meta analysis of genome-wide association studies for CAD (>22,000 CAD cases & >64,000 controls), we identified the COL4A1/COL4A2 locus as one of 13 novel regions associated with CAD (Nature Genetics 2011). The index SNP at this locus (rs4773144) has a minor allele frequency of 0.4 and is associated with an increased risk of CAD (allele specific odds ratio=1.21 in OHS). In a search for functional genetic variants, we resequenced the bidirectional COL4A1/COL4A2 promoter in 500 CAD cases and 500 controls and identified four novel SNPs, in promoter/enhancer regions essential for COL4A1 and/or COL4A2 gene expression and in strong linkage disequilibrium with several OHS risk SNPs. In a luciferase assay, the risk allele for the rs35466678 SNP, in the 5'UTR of COL4A2, reduced promoter activity by 13% ($p<0.005$). Furthermore, the linked risk alleles for the rs7327528 and rs117410570 SNPs, both within an enhancer essential for COL4A2 transcription, reduced COL4A2 promoter activity by 17% ($p<0.0005$). Reduced COL4A2 promoter activity in vitro due to these SNPs will be investigated further to determine effects on protein levels and basement membrane structure/function. By further analysis of the bidirectional promoter we identified a novel regulatory mechanism for the transcription of COL4A1 and COL4A2. Luciferase assays of promoter constructs in HT-1080 fibrosarcoma cells revealed that human collagen krox protein (hcKrox), previously shown to be involved in regulation of type I collagen, upregulates both COL4A1 and COL4A2 transcription in a dose-dependent manner. Quantitative RT-PCR studies are underway to determine the effect of overexpression of hcKrox in HT-1080 cells on endogenous COL4A1 and COL4A2 mRNA levels. Complementary studies will be conducted to characterize the binding of hcKrox to the COL4A1/COL4A2 promoter region and determine effects on protein expression. The discovery of hcKrox's effects COL4A1 and COL4A2 adds another piece to the puzzle of type IV collagen regulation. These findings are important because misregulation of COL4A1 and COL4A2 could have important consequences relevant to CAD, including effects on basement membrane integrity and angiogenesis.

**P-14**

## PET Imaging of avß3 Integrin Receptors Using a c(RGDyK) Peptide Labeled with [18F]FPyKYNE via "Click Chemistry"

**Ana Carola Valdivia (Supervisor: Dr. Jean N. DaSilva)**

**Introduction**: Integrin avß3 receptors are important biomarkers of angiogenesis, these cell surface receptors bind to peptides containing the amino acid sequence arginine-glycine-aspartic acid (RGD) present in the extracellular matrix. Integrin receptors are over expressed in ischemic tissues, atherosclerotic lesions and tumor vasculature. Here we present the radiolabeling of the azido-c(RGDyK) done with 2-[18F]fluoro-3-pent-4-yn-1-yloxypyridine [18F]FPyKYNE(1) via click chemistry in an automated dual reactor module TRACERlab FX N Pro (GEHealthcare) for the imaging of avß3 integrin receptor.
**Methods**: The precursor (2-nitro-pyridine alkyne) in DMSO is added to the reactor-1 containing K[18F]F/K222 and heated at 120? C for 10min. After cooling, the reaction mixture is transferred onto 3 silica cartridges connected in series and the [18F]FPyKYNE is eluted (pentanes/ether, 1/1) into the second reactor. After drying reactor-2, azido-c(RGDyK) in water, CuSO4 , sodium ascorbate and DMSO are added to a final water/DMSO ratio of 1/1, and heated at 65 °C for 25 min. The reaction mixture was purified by semi-prep reverse phase HPLC (Phenomenex, Luna C-18, 10µ). The fraction containing the [18F]-c(RGDyK) peptide is collected and the solvent evaporated under vacuum at 85°C and reformulated in saline. The identity and purity was assessed by analytical HPLC (Phenomenex, Luna C-18, 10µ) and by co-injection with the reference peptide. The reference

peptide F-c(RGDyK) was synthesized using the same synthetic procedure as described for the hot synthesis, and characterized by ESI-MS. Calcd for C35H47N12O9 [M+H]: 958.443; found: 958.446.
**Results**: [18F]c(RGDyK) was produced in two step radiosynthesis via click chemistry in an overall yield of 15-20% radiochemical yield (decay-corrected yield) and > 99.5% radiochemical purity in less than 120 min from end of beam (EOB).
**Conclusions**: A simple fully automated synthetic route for the preparation and purification of [18F]c(RGDyK) is described here for the potential imaging of avß3 integrin receptor. Initial biodistribution studies done with [18F]c(RGDyK) displayed uptake in aorta of -/- ApoE-knockout mice, demonstrating that [18F]c(RGDyK) has potential use in atherosclerosis.

**P-15**
**Vestigial-like 3 Regulates Myosin Light Chain and Skeletal a-Actin Promoters**
**Brian Cheng (Supervisor: Dr. Alexandre Stewart)**

**Background:** Drosophila Vestigial (Vg) interacts with Scalloped protein to potentiate the indirect flight muscles development. Recently, one of the four mammalian Vg homologs, Vestigial-like-3 (Vgl-3), was found to be associated with the myogenic lineage during mouse embryonic development. However, the function of Vgl-3 is not known.
**Results:** Mouse multi-tissue Northern blot showed two transcripts at 1.5 kb in the lung and 3.2 kb in the heart. During muscle differentiation, Vgl-3 mRNA level remained constant but its protein level decreased. Like Vgl-2, Vgl-3 also interacted with MEF2, TEAD1 and TEAD4 muscle-specific transcription factors in mammalian two-hybrid assays. Functionally, the co-expression of Vgl-3 and MEF2 co-activates an MEF2-dependent promoter. Vgl-3, however, repressed the mouse skeletal a-actin (ACTA1) promoter activity by 5-fold. To identify novel Vgl-3 interactors, we exploited a bioinformatic approach and discovered Ying Yang 1 (YY1) as a plausible candidate. Immunoprecipitation revealed a physical interaction between Vgl-3 and the ACTA1 repressor, YY1.
**Conclusions:** Vgl-3 is a transcription cofactor that may have a role in regulating a series of muscle-specific promoters by interacting not only with TEAD and MEF2 transcription factors but also with the multifunctional YY1 protein.

**P-16**
**Evaluation of a Collagen Matrix for Generation of an Angiogenic Population and as a Vehicle for Cell Delivery in a Porcine Model of Myocardial Hibernation**
**Céline Giordano (Supervisor: Dr. Marc Ruel and Dr. Erik J Suuronen)**

**Introduction:** Biomaterials with the ability to augment regenerative responses following an ischemic event are highly sought after. In this study, we examine the angiogenic potential of circulating progenitor cells (CPCs) grown on a collagen-based matrix, and test this matrix for CPC delivery in a porcine model of myocardial hibernation.
**Methods:** Human CPCs were isolated and cultured for 4 days on fibronectin or collagen/chondroitin sulfate blended matrices. CPCs were harvested and assessed for their adhesion and migration potential, and their ability to support tubule formation in an angiogenesis assay. Myocardial ischemia was induced in 28 pigs by ameroid constrictor placement around the LCx. Two weeks later, animals underwent echocardiography, dynamic rest and dipyridamole-induced stress NH3 PET imaging, followed by a rest FDG PET viability scan. The imaging protocol was repeated at follow-up. At week 3, animals were randomized to intramyocardial injection of PBS (control), CPCs, or CPCs+matrix. Animals were sacrificed at week 8. Adhesion of matrix-grown cells was 4-fold greater than fibronectin-grown cells. Matrix-raised CPCs also demonstrated increased migration (by 40%) into a 3D-polymer, augmented total length of capillary-like networks (by 18%), and a 1.9-fold increase in incorporation into tubules in vitro. Hibernation, defined as segmental mismatch >5% was significantly higher in the LCx than in the LAD territory (p=0.003). Myocardial blood flow (MBF) at stress and myocardial flow reserve (perfusion at stress/perfusion at rest) were significantly reduced in the LCx compared to the LAD (0.96 ± 0.07 vs. 1.97 ± 0.24, p=0.0002 and 0.99 ± 0.1 vs. 1.74 ± 0.28, p=0.03). There was also a correlation between increased MBF at stress, reduced mismatch score, and increased EF. Perfusion at stress in the LCx segment positively correlated with alpha smooth muscle actin staining for arterioles (r=0.7; p=0.02). Randomization is ongoing for 28 animals but groups will be unblinded at the end of April 2011. Improvement in LV function, myocardial perfusion, myocardial viability, and vascular density is expected to be the greatest in the CPCs+matrix group, mediated mainly through paracrine effects.
**Conclusions:** Our collagen matrix was successful in generating a more potent angiogenic cell population. In addition, this porcine model successfully demonstrated hibernating myocardium as detected by PET and is suitable for pre-clinical evaluations of angiogenic cell therapies. Observing increased benefits of cell+matrix transplantation in this relevant pre-clinical model would be a promising step towards global improvement of regenerative angiogenic therapies.

**P-17**
**The Use of 18F-FDG to Detect Atherosclerotic Lesion Changes in Mice**
**Lyne Sleiman (Supervisor: Dr. Robert S. Beanlands)**

**Background:** F-18-fluorodeoxyglugose (F18-FDG) is a glucose analogue and has been shown to be taken up by active macrophages in the atherosclerotic plaque. Active macrophage, such as the ones found in atherosclerotic lesions, have increased metabolic rate. Using 18F-FDG as a marker of monocyte metabolism and plaque inflammation, we aim to determine whether FDG PET could be used to monitor atherogenesis in mice using micro-positron imaging tomography (uPET) as well as computed tomography (CT) in order to monitor disease progression.
**Methods and Results:** Ten male C57BL/6 mice were used for this study. At 8 weeks of age, 5 mice were put on a high-fat high-cholesterol diet (HFD) for 12 weeks to induce atherosclerotic lesions and 5 mice were put on a regular chow diet for 12 weeks as controls. Following the HFD period, mice were given 1mCi 18F-FDG while under 1.5% isoflourane. They were scanned 1hr later using a uPET scanner for 30mins followed by a 9min CT scan. Aorta were dissected and underwent a 15min uPET scan followed by autoradiography imaging. Different organs including heart, ascending

aorta, branches and descending aorta were counted with a gamma counter for biodistribution. Autoradiography images show significant uptake in the aortic lesions corresponding to lesions seen on light microscopy with a correlation coefficient of r2 = 0.83. uPET scans of excised aorta show uptake in atherosclerotic lesions as confirmed by light microscopy images. Biodistribution data shows a statistically significant increase in 18F-FDG uptake in the aorta of mice fed a HFD compared to the control group fed a chow diet. Average of the % injected dose per gram of tissue (%ID/g) ± SDEV for aortic branch was 12.56 ± 6.48 vs. 2.6 ± 2.6 (p = 0.05) for HFD vs. chow fed mice; aortic arch uptake was 11.47 ± 3.38 for HFD vs. 3.56 ± 2.8 (p = 0.011) for chow fed mice; and abdominal aorta was 23.47 ± 5.5 for HFD vs. 7.66 ± 3.9 (p = 0.004) for chow fed mice. Mouse in vivo uPET scans show promising 18F-FDG uptake in atherosclerotic lesions in the aorta of HFD mice.
**Conclusions:** The use of 18F-FDG to image atherosclerosis shows a promising future for use in imaging disease progression and can be extended further to monitor therapy.

### P-18
### Right Ventricular Metabolic Imaging in Experimental Pulmonary Artery Hypertension

**Stephanie Thorn (Supervisors: Dr. Jean N. DaSilva and Dr. Michael Gollob)**

**Background:** Pulmonary artery hypertension (PAH) is a disease of progressive vascular remodeling, vasoconstriction, and right heart failure (HF). There is heterogeneity in the development of right HF, and the mechanisms and predictors remain largely unknown. It has been suggested that alterations in cardiac metabolism may be related to progressive RV dysfunction. This study was designed to evaluate the changes in fatty acid and glucose metabolism with cardiac PET imaging in experimental PAH.
**Methods/Results:** Monocrotaline was given as a single injection (70 mg/kg) to adult Fischer rats to induce PAH. Glucose and fatty acid metabolism was assessed with FDG and FTHA PET imaging four weeks after injection and reported as a standardized uptake value (SUV). Cardiac and pulmonary metabolism was correlated with RV size, cellular markers of metabolism and pulmonary smooth muscle cell proliferation. PAH was associated with a significant increase in cardiac size (heart weight:body weight ratio 0.0026 vs. 0.0036 normals vs. PAH, p=<0.01). Rats with PAH had significantly greater RV (SUV 4.2 vs. 3.3, p<0.05) and lung FDG uptake (SUV 1.3 vs. 0.6,p<0.05). Fatty acid metabolism was also increased in the RV of rats with PAH. (SUV 2.3 vs. 0.9, p<0.05) MCT induced PAH was associated with increased smooth muscle cell proliferation in the pulmonary arteries of PAH rats.
**Conclusions:** PAH is associated with metabolic changes in both the right ventricle and the lungs in a monocrotaline model of PAH. Further research is needed to determine the pathophysiologic relationship between a metabolic shift and the progression of PAH, and to determine the role of metabolic imaging in diagnosis and prognosis of PAH and right heart failure. Such studies are now ongoing.

### P-19
### A 3D Partial Volume Correction Strategy for Quantitative Cardiac Mouse PET Imaging

**Tyler Dumouchel (Supervisor: Dr. Rob deKemp)**

**Objective:** Quantitative cardiac PET imaging is limited by spatial resolution. In this study a 3D partial volume correction (PVC) algorithm is developed for mouse LV myocardial imaging.
**Methods/Results:** A 3D model of the ECG-gated cardiac geometry and activity was estimated and convolved with the scanner PSF. Regional parameters of the model (LV myocardium, blood and background activity, and epi/endocardial wall borders) were varied to fit measured image data. ECG gated cardiac mouse PET images, with and without noise, were simulated using the MOBY phantom convolved with a Gaussian PSF. Images were simulated with axial pixel sizes of 0.2 and 0.8 mm and resolutions of 1.25 and 1.8 mm. Images were generated with the LV oriented axially and at 20º off-axis. The algorithm was evaluated in six healthy mice injected with 18F-FDG, scanned with the Inveon and reconstructed with 8 ECG gates. The PVC reduced bias in LV myocardial activity from 35% to within 5% and decreased the COV from 11–12% to 6–9% on the simulated images, demonstrating the ability to simultaneously improve recovery and homogeneity. With the heart oriented on-axis, an axial resolution of 1.8 mm did not affect the corrected activity, however an axial pixel size of 0.8 mm caused a 3% underestimation (p <0.01) of activity, likely due to undersampling at the apex. Off-axis orientation of the heart did not affect corrected activity. Typical noise levels did not affect bias in corrected activity with the larger axial pixel size or resolution, although it did have an effect (p<0.05) when the heart was oriented off-axis. The PVC increased recovery in the gated mouse hearts by 30% and the COV was improved by 3% on average, demonstrating that the PVC performed comparably to the simulations.
**Conclusions:** PVC is necessary to restore quantitative accuracy in cardiac imaging. The 3D PVC algorithm explored in this study appears to be a feasible solution to improve quantitative accuracy in mouse heart imaging with PET.

### P-20
### Myocardial Blood Flow And Cardiac Angiotensin II Type 1 Receptor Expression in a Rat Model of Transient Ischemia

**Kumiko Mackasey (Supervisor: Jean N. DaSilva )**

**Introduction:** Cardiac Angiotensin II type 1 receptor (AT1R) expression has been shown to increase following ischemic injury induced by LAD ligation, a commonly used animal model of myocardial infarction. Non invasive imaging of receptor expression in the infarct area using PET is limited by reduced blood flow to the infarct zone. It is proposed that transient LAD ligation followed by reperfusion will elicit increased AT1R expression with maintained myocardial blood flow (MBF) in the area at risk.
**Methods:** Animal Model Male Sprague Dawley rats were separated into sham, permanent occlusion, 3min, 5min and 20min transient occlusion groups. For sham surgeries, the heart was exposed and the chest cavity was closed. Permanent occlusion involved liagating a 6-0 suture around the proximal end of the LAD. Transient ischemia was induced by temporary occlusion of the proximal LAD using a 6-0 suture secured around polypropylene tubing. The ligature was secured for 3, 5, or 20 min and released. Occlusion and restoration of blood flow was confirmed by blanching and recovery of myocardial tissue. PET Rats (n=30) were injected with 2.5-3 mCi of [13N] ammonia and scanned for 30 minutes to assess MBF at baseline or

approximately 2 weeks after surgery. Image Analysis FlowQuant© automated analysis software was used to create polar maps of the left ventricular myocardium tracer flow values.
Protein Expression: A separate group of animals (n=26) were divided into the groups mentioned above and underwent surgeries as previously described. Two weeks after surgery, animals were sacrificed and LV infarct zone was dissected and flash frozen. AT1R expression was assessed by Western blotting.
**Results:** For each animal, MBF in the LV segment within the LAD supplied area with the lowest flow was compared to a remote segment. Ratios of the flow in these segments were similar in baseline, 3min, 5min and 20min transient ligation groups. As expected, permanent ligation caused a significant decrease in this ratio when compared to baseline. While 3min and 5min ligation groups showed similar AT¬1R expression to sham, permanent occlusion and 20min occlusion produced an 88.45% and 63.88% increase respectively.
**Conclusions:** Whereas permanent LAD occlusion caused a decrease in MBF in the infarcted area as assessed by [13N] ammonia PET scan, transient ligations displayed similar flow to baseline values. The MBF data combined with the significant increase in AT1R expression in 20min ligation group suggests AT1R imaging may be possible in this animal model. In vivo serial measurements with PET radioligands to assess the area at risk will aid in understanding the progression of disease and to guide therapy non-invasively.

### P-21
### Parkin Functions in a Novel Vesicular Pathway Governing Mitochondrial Quality Control

**Vincent Soubannier (Supervisor: Dr. Heidi McBride)**

Interference with the mechanisms that govern mitochondrial quality control have been linked with human diseases, including neurodegenration or heart failure. However, the mechanisms that regulate mitochondrial protein and lipid turnover are poorly understood. We have identified a novel mitochondrial quality control pathway characterized by mitochondria derived vesicles (MDVs). MDVs were induced by oxidative stress and delivered to lysosomes, as monitored by confocal microscopy. Their biogenesis can be reconstituted in vitro and is independent of DRP1-induced mitochondrial fission. Remarkably, the amount of cargo removed by the MDV pathway is comparable to the rates of degradation mediated by mitochondrial proteases. MDVs are selectively enriched for oxidized proteins, whose identity was dependent upon the type of mitochondrial stress. In particular, the induction of MDVs by intrinsic mitochondrial damage required the selective recruitment of Parkin to the vesicles. These data are the first to characterize a novel vesicle transport route between the mitochondria and lysosomes, providing new insights into the pathogenesis of Parkinson's disease.

### P-22
### Changes in the Mechanical Properties of the Artery Wall with Over-expression of Heat Shock Protein 27: Clues to Understanding Lesion Stability?

**Charles Cuerrier (Supervisor: Dr Edward O'Brien)**

**Introduction:** The O'Brien laboratory discovered that elevated serum levels of Heat Shock Protein 27 (HSP27) attenuate experimental atherogenesis in an ApoE-/- mouse model. Indeed, the over-expression of HSP27 (ApoE-/-HSP27o/e) reduces aortic lesion area as well as lipid and free cholesterol content in the intima. Atherosclerotic lesion development is also associated with arterial stiffening; however, advanced plaques may be prone to rupture resulting in arterial occlusion and ischemic syndromes. The aim of the present study was to examine the effect of HSP27 over-expression on the mechanical properties of the thoracic aorta in a mouse model of atherosclerosis, with a special emphasis on vascular stiffness.
**Methods/Results:** In collaboration with the Pelling laboratory, we developed an apparatus that radially stretches an arterial cross-section at a constant rate while vessel tension is recorded with a force transducer. The mechanical properties of the thoracic aortae of 1-year old ApoE-/- and ApoE-/-HSP27o/e mice fed a normal chow diet were studied. Briefly, thoracic aortae were carefully removed and transferred into a Krebs' solution maintained at 37o C. Loose connective tissue was carefully removed and the aortae were cut into 2-mm rings. Each ring was suspended horizontally between 2 wire hooks in an organ bath. The experiment consisted of 12 stretch cycles of preconditioning followed by one experimental stretch run from 0% to 100% strain. To determine arterial stiffness, ring length and wall thickness were studied before each stretching experiment. The histological composition of vessel wall was also independently assessed. Whereas there was no significant difference between the two animal groups regarding the vessel wall thickness, the Young's modulus (a material property that describes its stiffness) was increased by 40% in the aortic root ($p<0.01$) and 24% in the first part of the thoracic aorta ($p<0.01$) in ApoE-/-HSP27o/e compared to ApoE-/-mice. These results are consistent with the histological observation of an increased number of smooth muscle cells and an accumulation of collagen fibers in the artery wall with HSP27 over-expression.
**Conclusions**: In addition to increasing the amount of smooth muscle cells and collagen fibers, HSP27 over-expression increases the aortic wall stiffness in ApoE-/- mice. These data provide new clues as to how HSP27 may result in improved atherosclerotic lesion stability and perhaps the mitigation of plaque rupture. To better characterize this phenomenon, additional studies of the vessel wall mechanical properties are ongoing using an accelerated mouse model of atherosclerosis and the administration of recombinant HSP27, a novel therapeutic.

### P-23
### Pharmacokinetics of a recombinant Atrial Natriuretic Factor Fusion Protein

**Cody Sarch (Supervisor: Dr. Mercedes L. Kuroski deBold)**

**Introduction:** Natriuretic peptides are polypeptide hormones produced, stored and released by the atria of the heart that causes increased sodium and water excretion by the kidney, inhibits the rennin angiotensin aldosterone system (RAAS) and stimulates vasodilation. These effects are beneficial for patients with congestive heart failure and hypertension. However, with a half-life of 1-2 min, treatment using ANF is very expensive, for example iv delivery in hospital.
**Purpose:** To produce a recombinant HSA-ANF with a longer half-life and similar biological affects as native ANF.
**Objective:** To test if the HSA-ANF induced increase in plasma cGMP, a marker of ANF's biological effects, is correlated with a decrease in mean arterial blood pressure (MAP).

**Materials and Methods:** We have fused ANF to human serum albumin (HSA) in a Pichia pastoris expression system, cultured, IMAC (Immobilized metal ion affinity chromatography), HPLC purified and injected into Cd1 male mice in a dose response fashion. The HSA-ANF biological effects were monitored by measuring changes in MAP by tail sphygmomanometer. Plasma cGMP was determined by EIA.
**Results:** In vivo administration of HSA-ANF showed increasing plasma cGMP levels with increasing dosage (3.8x10-6M, 5.8x10-6M and 7.8x10-6M) injections. These circulating levels remained elevated for up to 360 min as compared to 5 min for the ANF injection (7.3x10-8M). MAP decreased as expected in a dose related fashion.
**Conclusions:** In vivo HSA-ANF showed no side effects, a longer half-life than native ANF and similar biological actions as ANF. HSA-ANF is a promising new drug for congestive heart failure and hypertension.

## CLINICAL SCIENCE POSTER PRESENTATIONS

### P-24
### Use of Bilateral Internal Thoracic Artery During Coronary Artery Bypass Graft Surgery in Canada: The BITA Survey

Stefano Mastrobuoni (Supervisor: Dr. Fraser D. Rubens)

**Background:** The Internal Thoracic Artery (ITA) is the gold-standard conduit in CABG surgery. The use of the left ITA (LITA) to the LAD is associated with significantly better outcomes compared to vein grafts. Therefore the use of the right ITA may add further benefit and in fact there is increasing clinical evidence of improved results with the use of bilateral ITAs (BITA) as compared to single ITA (SITA). However a recent report of the Society of Thoracic Surgery revealed that only a very small percentage of patients receive BITA in North America. The aim of this study was to determine the current use of BITA during CABG surgery amongst cardiac surgeons in Canada and identify the main concerns that limit the use of these conduits.
**Methods:** We developed an on-line survey with 17 questions about the use of BITA in different clinical scenarios. An invitation to participate was sent to all the adult cardiac surgeons currently in practice in Canada. Surgeons were questioned in order to figure out how often they use BITA and to identify concerns and limiting factors to a widespread use of these arterial conduits.
**Results:** One hundred and one surgeons (69%) out of 147 currently in practice across 27 different hospitals completed the survey. Forty percent of surgeons use BITA only sometimes (6-25% of cases), 37% very infrequently (<5% cases), 16% often (26-50%) and only 7% very often (>50%). The most common concerns to the use of BITA are the risk of sternal wound infection and the unknown superiority of RITA over other conduits. We found no correlation between the years in practice and the use of BITA but junior surgeons were significantly more concerned about the risk of sternal wound complications whereas senior surgeons were not convinced of the superiority of BITA. Furthermore, BITA frequent users do not consider diabetes and age a contraindication to the use of BITA as opposed to the infrequent users.
**Conclusions:** The majority of Canadian cardiac surgeons consider few clinical features such as IDDM or morbid obesity as contraindications to the use of BITA,. However the reported use of BITA is low. The main concern is the risk of sternal wound complications that has to be balanced with the benefit of two arterial grafts. A wider diffusion of this technique is warranted to improve the results of coronary surgery.

### P-25
### Assessment of Left Ventricular Diastolic Function Using Cardiac Computed Tomography

Mustapha Kazmi (Supervisor: Dr. Benjamin J. Chow)

**Background:** Heart failure is a leading cause of cardiac morbidity and mortality, with diastolic dysfunction being the etiology in approximately 50% of patients. While echocardiography is currently the preferred imaging modality to detect diastolic dysfunction, the diagnosis is often challenging due to complex algorithms and the dependence of the measured parameters on filling pressures. As an emerging imaging technology, we sought to determine if cardiac computed tomography (CT) can be used to assess diastolic function.
**Methods/Results:** We identified 25 patients with an echo-cardiographic diagnosis of diastolic dysfunction who underwent a retrospectively-gated CT. Using CT we calculated several parameters to assess diastolic function, including Peak Filling Rate, Mitral Inflow Velocities, Myocardial Velocities, and Left Atrial Volume. These parameters were also measured in 25 matched control patients. The ratio of peak filling rate to end-diastolic volume did not differentiate patients with diastolic dysfunction to controls ($p > 0.05$). Mitral inflow velocities (E and A) determined by CT did not correlate well with the echocardiography measures, and could not be used to differentiate patients with diastolic dysfunction from controls ($p > 0.05$). Mitral annular velocities (e') measured by CT did not correlate well with the corresponding echo measurements, and also could not be used discern patients with diastolic dysfunction from controls ($p > 0.05$). Despite cardiac CT being more accurate for left atrial volume measurements, this parameter did not differentiate those with abnormal function and controls ($p > 0.05$).
**Conclusions:** Retrospectively-gated cardiac CT is not a viable imaging modality to assess left ventricular diastolic dysfunction.

### P-26
### Stress Thallium Myocardial Perfusion Imaging Using a New Dedicated Solid State Cardiac SPECT Camera Compared to Conventional SPECT

Vikas Tandon (Supervisor: Dr. Terrence D. Ruddy)

**Background:** ECG-gated single photon emission computed tomography (SPECT) has been a standard method of investigation for the purposes of cardiac risk stratification, diagnosis and prognosis. Traditionally, cardiac SPECT has been performed using standard dual-head gamma cameras with parallel hole collimators. Recently, dedicated cardiac cameras using cadmium zinc telluride (CZT) solid-state detectors and pinhole collimation have been developed and provide greater sensitivity as well as better energy and spatial resolution when used with 99mTc tracers. We report our first experience with this new technology using Thallium-201.
**Methods/Results:** We scanned 50 patients who were intermediate to high risk for coronary artery disease (CAD) using our traditional dual-head SPECT camera (Infinia, GE Healthcare) as well as our new

solid state CZT camera (GE Discovery NM 530c.) Patients underwent standard 1 day stress/redistribution protocol using Thallium-201. Images on the CZT system were acquired immediately following the images on the traditional camera. Images were randomized and blindly analyzed by 2 experienced nuclear cardiology physicians using 4DM SPECT on a HERMES workstation. Images were analyzed visually for sum stress/sum rest scores (SSS/SRS) as well as regional wall motion abnormalities. Correlation co-efficients were calculated for SSS and SRS between the two cameras and the presence of ischemia was assessed.
Among the 50 patients, the Pearson co-efficient for correlation between the two cameras was 0.90 for SSS and 0.88 for SRS ($p<0.001$ for both). Assessment of ischemia as indicated by the summed difference score suggests a trend toward more apparent ischemia with CZT than with the traditional camera. This trend may be related to improved resolution of the CZT system or differences in the appearance of attenuation artifacts as has been seen with 99mTc labeled tetrofosmin studies.
**Conclusions:** Preliminary data show excellent correlation in summed stress and rest scores between images taken using standard SPECT gamma cameras and our new solid state CZT camera. The differences in ischemia suggest great sensitivity for obstructive CAD with the new technology and will require further investigation.

### P-27
### Standards and Quality Assurance for the Multicenter PET Rubidium ARMI (Alternative Radiopharmaceutical for Myocardial Imaging) Trial

**Jennifer Renaud, Cardiac PET, Cardiac Imaging Research Analyst**

**Background:** The globally reduced supply of Tc-99m motivates investigation of alternatives to SPECT myocardial perfusion imaging (MPI) for assessment of coronary artery disease (CAD). Rb-82 PET is considered to have superior accuracy and lower radiation dose for MPI. Rb-ARMI is a multicentre imaging study with an initial objective of disseminating and standardizing Rb-82 PET MPI with highly repeatable interpretation in Canadian centres using 3D PET-CT technology.
**Methods/Results:** Imaging protocols and quantitative polar-map scoring were standardized via rest and stress qualifying phantom scans at the core and recruiting sites. Subsequently, patients underwent low-dose (10 MBq/kg) rest and dipyridamole stress Rb-82 PET MPI. Sum stress & rest scores (SSS, SRS) [0–4], and sum difference scores (SDS=SSS-SRS) were visually assessed using a standardized color-map and 17-segment model (4DM, INVIA).The first 25 cases from the core and recruiting sites were co-read at the core lab to assess the variability of SSS and SDS, and clinical diagnosis between the sites and core. Pearson's correlation was used to assess statistical agreement. Scores with differences > 3 underwent a third review to reach consensus. Phantom scans consistently resulted in the expected scores of SSS=2, SRS=0, SDS=2 at all imaging sites with 6 different 3D PET-CT cameras. Comparison of clinical patient scores between the core and three of the recruiting sites resulted in good overall agreement: Pearson's R = 0.93 for SSS and 0.92 for SDS. 85% of SSS scores and 89% of SDS scores had differences (site-core) = 3. The largest discrepancies occurred in cases with large defect regions; however, these cases were all correctly identified as abnormal. Following consensus review the overall agreement improved to: Pearson's R = 0.96 for SSS and 0.95 for SDS (Figure 1). 51% of cases were diagnosed as abnormal by core and site reviews, indicating 100% consistent clinical interpretation.
**Conclusions:** Good agreement was found between scoring of the Rb-82 MPI scans at the core and recruiting sites. Agreement was further improved with consensus reading of the most discrepant cases, demonstrating improved scoring consistency with increased training and experience. This indicates that standardized and repeatable interpretation is achievable across imaging centers with different 3D PET-CT scanners, using these imaging standards and quality assurance methods.

### P-28
### In Patients with Normal Relative Perfusion Imaging, is there a Relationship Between Calcium Score and Myocardial Flow Reserve?

**Ilias Mylonas (Supervisor: Dr. Robert S. Beanlands)**

**Introduction:** Standard perfusion may underestimate the real extent of disease and may be unable to detect atherosclerotic microvascular dysfunction. We have shown that noninvasive quantification of myocardial flow reserve (MFR) using cardiac positron emission tomography (cPET) has prognostic value independent of relative normal myocardial perfusion imaging (MPI) even when relative MPI is normal. Likewise the calcification in coronary arteries measured as the Agatston score (AS) on cardiac computed tomography (CCT) represents a valuable tool for stratification of patients with suspected coronary artery disease (CAD) and can be considered a quantifiable marker of disease. Our objective was to determine the relationship between AS and MFR and whether AS can predict reduced MFR (<2,5) in patients with normal MPI on cPET (i.e. a sum stress score (SSS) < 4).
**Methods/Results:** 101 patients (mean age = 60.9 +/-10.4; 52 males) without known CAD, who underwent cPET MPI, and had a SSS < 4, and underwent a CCT calcium score within six months of the cPET scan were included. Patients with prior coronary artery bypass, percutaneous intervention, valvular disease, or a pacemaker were not included. Patients were categorized according to normal MFR (>2.5 ml/min/g) or abnormal MFR (<2.5 ml/min/g). Spearman correlation showed a weak but significant inverse correlation between calcium score and MBF (figure 1, r = -0.253; p=0.0109). When patients were divided into 3 groups based on AS (figure 2, group 1: 0 (n=34); group 2: 1-400 (n=38); group 3: >400 (n= 29)) there was a significant difference in MFR (3.30+/- 0.98; 2.84 +/- 0.85; 2.63 +/- 0.79, respectively; p=0.022, ANOVA; p =0.010 group 1 vs 3). A calcium score of 0 had a sensitivity of 32/37 (87%) and specificity of 29/64(45%) for predicting a MFR < 2.5 ml/min/g.
**Conclusions:** In patients with normal relative MPI, AS and MFR have a significant, however weak negative correlation. Patients with low AS(=0) have significantly higher MFR than those with high AS > 400. AS was sensitive but not specific for reduced MFR. The wide variation in MFR at different levels of AS suggests that in patients with low risk relative MPI on cPET, AS and MBF provide different yet complimentary information. Larger prospective outcome studies are required to determine the incremental prognostic value of these parameters.

**P-29**

## Prevalence and Clinical Significance of Functional Mitral Stenosis after Mitral Valve Repair for Myxomatous Mitral Regurgitation

Kristen Chen (Supervisor: Dr. Kwan Chan)

**Background:** Mitral valve repair (MVr) has become the standard treatment for most cases of severe myxomatous mitral regurgitation (MMR); however, some patients have exhibited evidence of significant mitral stenosis (MS) after MVr. The aim of this study was to assess the prevalence and clinical significance of this type of MS following successful MVr.
**Methods:** 85 patients who had MVr for MMR in 2001-2009 were recruited and assessed with bicycle stress echocardiogram, 6 minute walk test, SF-36 health survey and BNP measurements. We excluded patients with significant residual MMR, aortic valve disease or ventricular dysfunction. The age was 69 ± 20 yrs; 59 (69 %) were male; 83 (98 %) had mitral annuloplasty with 57 (69 %) receiving a band and 26 (31 %) a full ring. In this study, MS was defined as a resting mean mitral gradient (MMG) > 4 mmHG.
**Results:** 36 (42 %) patients met the criteria for MS; they had higher resting and peak exercise right ventricular systolic pressure (RVSP) and reported worse general health with limited exercise capacity. MS was associated with the use of a full ring during MVr ($p < .0001$). MS patients had elevated MMG at rest which correlated strongly with elevated peak exercise MMG ($r = 0.74$, $p < .0001$).
**Conclusions:** MS post MVr is common problem associated with worse general health status and exercise capacity. Patients with MS develop greater elevations in MMG and RVSP during exercise, which can be readily assessed by bicycle stress echocardiogram. The risk of MS may be reduced by limiting the use of mitral rings.

**P-30**

## Preoperative Anemia is a Risk Factor For Mortality and Morbidity Following Aortic Valve Surgery

Elsayed Elmistekawy (Supervisor: Dr Munir Boodhwani)

**Objectives:** Anemic patients have been shown to have poorer outcome following non-cardiac surgery as well as coronary artery bypass surgery. However, the impact of anemia on patients undergoing aortic valve surgery has not been well studied. We sought to evaluate the effect of anemia on early outcomes following aortic valve surgery.
**Methods:** All patients undergoing non emergent aortic valve surgery (n = 2698) with or without other concomitant procedures between 1997 to 2010 were included. Preoperative anemia was defined as per World Health Organization guidelines as hemoglobin (Hb) <130 g/L in men and Hb<120 g/L in women. Multivariable analyses were used to determine the association between anemia and postoperative outcome.
**Results:** The prevalence of preoperative anemia was 32.2%. Patients with anemia were older (71+12 years versus 66+13, $p < 0.001$), more likely to have urgent surgery, recent MI, higher creatinine level and impaired preoperative left ventricular function. Overall unadjusted mortality was 2.8% in non-anemic patients versus 7.97 % in anemic patients. Anemic patients were more likely to require renal replacement therapy (11% versus 3%, $p < 0.0001$), and prolonged ventilation (24% versus 10%, $p < 0.0001$). Lower preoperative hemoglobin was an independent predictor of mortality (odds ratio 1.19, 95% CI: 1.04 – 1.34, $p = 0.007$) and composite morbidity (odds ratio 1.36, 95% CI: 1.05 – 1.77, $p = 0.02$) after AVR. (Table 1). Mortality and composite morbidity were significantly higher with lower levels of preoperative hemoglobin.
**Conclusions:** Preoperative anemia is a common finding in patients undergoing aortic valve surgery and is an important and potentially modifiable risk factor for post-operative morbidity and mortality.

**P-31**

## R in V1 as a Predictor of Response to CRT

Mark Perrin (Supervisor: Dr. David Birnie)

**Background:** Cardiac resynchronization therapy (CRT) reduces heart failure symptoms, improves left ventricular (LV) function, and decreases mortality in NYHA II to IV (ambulatory) heart failure with a wide QRS complex. Still, one third of patients derive no clinical benefit; substantially less demonstrate objective improvement of LV function. Previous studies have shown that a left bundle branch block (LBBB) pattern on the surface electrocardiogram is a strong predictor of response compared to the presence of a non-specific intraventricular conduction delay (NIVCD). Recent data has suggested that ECG features can further subdivide patients with 'classic' LBBB (defined according to current guidelines) into true LBBB and those with some residual left bundle conduction. We hypothesized that patients with residual left bundle conduction would have a lesser response to CRT.
**Methods/Results:** An r wave > 1mm in lead V1 (r-V1) or a q wave > 1mm in lead aVL (q-aVL) was used to identify patients with residual left bundle conduction. 40 patients were prospectively enrolled with LVEF = 35 %, NYHA II to III and a QRS duration of = 130 ms. Patients with atrial fibrillation or RBBB were excluded. Three groups were defined: those with LBBB without an r in V1 or q in aVL > 1mm (Group 1 – n = 12); those with LBBB with an r in V1 and/or a q in aVL > 1mm (Group 2 – n = 15); and those with NIVCD (Group 3 – n = 13). The baseline QRS duration was not significantly different between the groups. The mean reduction in QRS duration with CRT was - 8.0 ± 11.0 ms in Gp I, - 0.8 ± 8.24 ms in Gp II ($p = 0.06$), and 0.15 ± 8.0 ms in Gp III ($p = 0.04$ Gp I vs. Gp III). The mean improvement in LV EF was 11.9 ± 11.9 % in Gp I and 3.8 ± 5.4 % in Gp II ($p < 0.02$), and 2.5 ± 4.4 % in Gp III (p NS for Gp II vs. Gp III). The mean percentage reduction in LVESV was 26.4 ± 39.2 % in Gp I, 14.3 ± 22.9 % in Gp II (p NS), and 5.6 ± 17.3 % in Gp III (p NS) (All mean ± S.D).
**Conclusions:** In patients with a classic guideline-defined LBBB, the absence of an r wave > 1 mm in V1 or a q wave in aVL > 1mm (suggesting no residual left bundle conduction) was predictive of a marked improvement in left ventricular ejection fraction with CRT. Conversely, patients with LBBB and r-V1 or q-aVL responded less well. Confirming the results of previous studies, patients with NIVCD showed only small benefit with CRT. These intriguing results need to be tested in a larger sample size.

**P-32**

## Genetic Testing for CYP2C19*2 but not for PON-1 QQ Carrier Status Predicts High On-Clopidogrel Platelet Reactivity in Patients Undergoing Percutaneous Coronary Interventions

Sandro Goncalves (Supervisor: Dr. Derek So)

**Background:** High on-clopidogrel platelet reactivity (unresponsive-ness) is associated with major adverse clinical events (MACE) after percutaneous coronary intervention (PCI). Genetic factors may play a crucial role in the impairment of clopidogrel response. CYP2C19*2, a common variant allele has been associated with MACE post PCI. Recently, another variant PON-1 QQ has been proposed to be a major determinant of clopidogrel responsiveness. We sought to assess the relative strength of these polymorphisms to platelet response to clopidogrel.
**Methods:** Patients undergoing non-emergent PCI after a 600mg bolus of clopidogrel were sequenced for CYP2C19*2 and PON-1 QQ carrier status. Response to clopidogrel was measured in platelet reactivity units (PRU) using the Verify-Now P2Y12 assay.
**Results:** Of 88 patients enrolled, mean age was 59.5 ± 9.2 years, 79% were male, 24% had diabetes, 32% had hypertension and 38% were smokers. CYP2C19*2 was present in 15 (17%) and PON-1 QQ in 42 (48%) of patients. PRU was significantly higher among carriers of the CYP2C19*2 (194 ± 67 vs 127 ± 98, p= 0.01). There was no significant difference in PRU according to PON-1 QQ (128 ± 100 vs 148 ± 93, p = 0.32). By multivariate regression, the presence of the CYP2C19*2 allele was independently associated with high on-clopidogrel platelet reactivity (OR 7.53 95% CI 1.35 – 41.90, p = 0.02).
**Conclusions:** Our study confirms the association between the CYP2C19*2 allele and high on-clopidogrel platelet reactivity. However, PON-1 status was not associated with altered clopidogrel responsiveness. These results are important to future strategies for personalized anti-platelet therapy.

## ALLIED AND POPULATION HEALTH POSTER PRESENTATIONS

### P-33
### Implementation of Automatic Referral To Cardiac Rehabilitation: Predictors Of Participation

**Amy E. Mark (Supervisor: Dr. Robert Reid)**

**Background:** Cardiovascular disease (CVD) remains the leading cause of death in Canada. Cardiac rehabilitation (CR) is an essential component of secondary prevention of morbidity and mortality. Despite the known benefits of CR, it is a highly underutilized resource. As well, biases have been noted within the referral process whereby younger and males patients were more likely to receive a referral to CR. At the University of Ottawa Heart Institute (UOHI) measures have been taken to remove such biases through the implementation of automatic referral processes. The current study examined CR referral rates, and the predictors of CR referral and attendance at CR intake session.
**Methods/Results:** In 2008 an automatic referral to CR program was implemented at UOHI. Nurses systematically visited all patients admitted to UOHI and provided them with a referral to CR. Information was collected on age, sex, diagnosis, physician, location (unit) in UOHI for each patient. Attendance at CR intake sessions was recorded. Chi square analysis was used to compare CR participation between sex, diagnosis, and location in UOHI. Logistic regression was used to predict accepting CR referral and attendance at CR intake session. A total of 5666 patients were approached by the automatic referral nurses in 2008 and 2009. The rate of CR referral increased from 25% in the 2003/04 fiscal year to 72% in 2008/09. The proportion of males accepting referral was slightly higher, 47%, compared to females, 42% (P<0.01), and 83% of males and 75% of females accepting referrals attended intake. Younger patients were more likely to accept a CR referral. Acceptance of a CR referral differed by diagnosis; 70% aortic valve repair/replacement (AVR), 64% multiple procedure (e.g., AVR with coronary artery bypass graft; CABG), 63% CABG, 40% percutaneous coronary intervention (PCI), 34% angina and 25% coronary heart failure. Compared to CABG patients, AVR patients were 1.45 (1.06-2.00) times more likely to accept a referral to CR whereas PCI, angina, arrhythmia, catheterization, coronary heart failure, myocardial infarction, and other diagnoses were 0.37 (0.32-0.43), 0.31 (0.18-0.52), 0.13 (0.05-0.34), 0.14 (0.08-0.24), 0.22 (0.14-0.33), 0.41 (0.32-0.53), and 0.45 (0.34-0.60) less likely, respectively. Only MI (1.70, 1.05-2.76) and PCI (1.69, 1.32-2.17) patients were more likely to attend CR intake compared to CABG patients.
**Conclusions:** Automatic referral dramatically increase the proportion of patients receiving referral to CR however it still differs between sexes. The likelihood of accepting a CR referral and attending CR intake differs based on diagnosis.

### P-34
### Patient Satisfaction with an Interactive, Voice Response-Mediated, Follow-Up and Triage System for Smoking Cessation

**Ashley Armstrong (Supervisor: Dr. Robert Reid)**

**Background:** Interactive voice response (IVR) technology has the potential to improve follow-up with smokers after hospitalization and to enhance triage to clinical support for smoking cessation. The objective of the IVR Patient Satisfaction Questionnaire was to collect quantitative and qualitative feedback from patients regarding their experiences with the IVR system.
**Methods:** From July 2006 to October 2009, smokers admitted to the UOHI for ACS, PCI or diagnostic catherization were approached to participate in the IVR study and were randomly assigned to either (1) the usual care group; or (2) the IVR intervention group. Two-hundred and seventeen patients were randomized to the IVR group and received 8 automated telephone calls from the IVR system at 3, 14, 30, 60, 90, 120, 150 and 180 days after hospital discharge. The automated calls took the patient through a series of questions concerning current smoking status, confidence in staying smoke-free, and use of pharmacotherapy, self-help materials and other forms of cessation supports. If patients identified that they had resumed smoking or indicated that their confidence in remaining smoke-free was low, they were contacted by a smoking cessation nurse-counselor. Patients randomized to the IVR group were sent a questionnaire inquiring about their satisfaction with the IVR system.
**Results:** Patients in the IVR group had a mean age of 54 years and 76% were male. Completion rates for the IVR calls at 3, 14, 30, 60, 90, 120, 150 and 180 were 82%, 87%, 86%, 86%, 81%, 76%, 79% and 72% respectively. Ninety-five IVR patients (44%) responded to the IVR Patient Satisfaction Questionnaire. Overall impression of the IVR system was favourable (79%). Patients were able to clearly hear (87%) and understand (86%) the automated system. Eighty-four percent reported the pace of the call to be just right (versus too slow or too fast), 81% reported the length of the call to be just right (versus

too short to too long) and 83% reported that they had enough time to answer the questions posed by the automated system.
**Conclusions:** Smokers with CHD were predominately in favour of an interactive voice response follow-up system to support smoking cessation post hospital discharge.

### P-35
### Varenicline Versus Nicotine Replacement Therapy for Smoking Cessation in Patients Hospitalized with Coronary Artery Disease: A Pilot Randomized Trial

**Debbie A. Aitken, APN, Smoking Cessation, Minto Prevention and Rehabilitation Center**

**Background:** No studies have compared varenicline versus nicotine replacement therapy (NRT) in smokers hospitalized with CAD. A pilot randomized trial assessed the feasibility of conducting a larger, superiority trial.
**Methods:** Fifty current smokers hospitalized for CAD were randomized to varenicline for 12 weeks (0.5 mg/day for 3 days; 0.5 mg twice daily for 4 days; 1 mg twice daily for 11 weeks) or transdermal NRT for 12 weeks (21 mg/day for 6 weeks; 14 mg/day for 4 weeks; 7 mg/day for 2 weeks). Participants received in-hospital counseling and telephone counseling 3, 14, 30 and 50 days after hospitalization. A priori indicators of feasibility were that: = 25% of eligible smokers would participate; treatment completion would be = 60%; study completion would be = 80%; and there would be = 6% between-group difference in abstinence rates at 26 weeks favoring varenicline.
**Results:** Of 1243 smokers, 456 were eligible, 50 (11.0% of eligible) were randomized, and 40 (80%) completed the study. Overall, 32 (64.0%) participants completed treatment, including 17 (68.0%) assigned to varenicline and 15 (60.0%) assigned to NRT. The verified 7-d point prevalence abstinence rate at 26 weeks was 24.0% in both treatment groups (OR = 1.00; 95% CI: 0.27 to 3.66; P = 1.000)
**Conclusions:** It is not feasible to conduct a superiority trial to compare varenicline to NRT in smokers hospitalized with CAD using the current design because there is no difference in efficacy between treatments. Varenicline's risk/benefit profile could be evaluated in an equivalence trial.

### P-36
### The Quit Smoking Program (QSP) at the University of Ottawa Heart Institute: Design, Patient Characteristics and Outcomes

**Debbie Aitken, APN, Smoking Cessation, Minto Prevention and Rehabilitation Center**

**Background:** Quitting smoking is the most important step that a smoker can take for cardiovascular health. Best practice guidelines recommend the use of first-line smoking cessation medications, strategic advice and follow-up support. The Quit Smoking Program (QSP) at the University of Ottawa Heart Institute is a nurse-managed program that assists smokers to quit using these recommended interventions. Here we describe the QSP, characteristics of smokers using the program, and smoking cessation outcomes achieved.
**Methods:** All smokers entering the program from December 2006 - 2009 were included in the analysis. Smokers attended an information session followed by individual appointments with a tobacco treatment nurse specialist at -2, +2, +5, and +10 weeks around a target quit date. Questionnaires including demographics, medical and psychiatric history, and smoking-related and motivational variables were completed. All participants received strategic advice tailored to their individual needs. The primary outcome measures included 7-day point-prevalence abstinence. Analyses were intention to treat; patients lost to follow-up were considered smokers.
**Results:** Participants (N = 876; mean age = 50.7(±11.2) years; 47% male) reported smoking on average 23 (±13.5) cigarettes per day. Mean age of first cigarette = 19.4 (±7.1) years. Co-morbidities included hypertension/dislipidemia (49.3%), respiratory (45.1%), cardiovascular (42.1%), depression (40.9%); gastrointestinal (33.8%); endocrine (24.4%), dermatology (23.5%); anxiety (23.1%); cancer (14.0%); Smokers reported a high level of importance to quit smoking at baseline session (M = 9.4; 0-10 scale), however their confidence level was lower (M = 6.8; 0-10 scale). First line medications used were nicotine replacement therapy (82.5%), bupropion (7.7%); varenicline (7.1%). On average attendance was 3 out of 4 recommended visits. The confirmed quit rate at the end of the QSP is 18%.
**Conclusions:** The QSP serves primarily smokers with long smoking histories and high levels of nicotine dependence. These participants frequently are already suffering from tobacco-related illnesses and /or psychiatric illness. Despite these challenges, the program has been able to achieve clinically important improvements in cessation outcomes.

### P-37
### Healthcare Professionals Knowledge and Attitudes about Smoking Cessation Before Implementation of the Ottawa Model for Smoking Cessation

**Jana Kocourek, Project Coordinator and Laura Jones, Data Analyst, Minto Prevention and Rehabilitation Center**

**Background:** In 2002, the University of Ottawa Heart Institute developed the Ottawa Model for Smoking Cessation (OMSC) – an institutional program that systematically identifies and documents patient smoking status, and provides treatment. Although healthcare professionals (HCP) are encouraged to address patients' tobacco use, the integration of cessation counseling into practice has not been consistent due to differences in attitudes, beliefs and knowledge about smoking cessation (SC). The objective of this project was to describe knowledge and attitudes of HCP's regarding SC before implementation of the OMSC clinical SC program.
**Methods/Results:** Staff surveys were created based on the Theory of Planned Behavior by the OMSC program evaluation specialist. Pre-implementation surveys were completed by 819 HCP's immediately before an OMSC training session at 23 hospitals in Canada. The OMSC program was implemented within one month of surveys being completed. Statistical analyses of outcomes were completed using descriptive statistics (frequencies) in SPSS statistical software. Eighty two percent of HCP's surveyed were nurses of which 88% did not use tobacco. Outcomes are presented in the table below.
**Conclusions:** A majority of hospital staff possess positive attitudes towards implementing a smoking cessation program in their

hospitals and believe it is the role of all patient providers (i.e. nurse, physician, and other HCP) to address smoking cessation with patients. Implementation of the OMSC program is ongoing and post implementation data results are forthcoming.

**P-38**

### Get With the Guidelines – 4 Years Later

**Lorraine Montoya, BSN, MAdEd, Regional Program Educator, Minto Prevention and Rehabilitation Center**

Get with the Guidelines, a program aimed at improving care for patients diagnosed with Acute Coronary Syndrome, was implemented throughout a regional health authority in 2007. Implementation was guided by established principles of knowledge translation; specifically, establishing baseline practices, defining target outcomes, tailoring key messages to the specific audience, embedding the new practices into usual care, and, finally, capturing outcome data and providing regular feedback to each partner institution.

The primary outcome identified in this initiative centred on the GAP tool, a nurse-driven discharge process designed to increase patient self-management and self-efficacy behaviours as articulated by the Chronic Disease Management Model. Embedded into the GAP tool are the best practices identified for management of Acute Coronary Syndrome.

As each institution within the region implemented, data that included use of the GAP tool along with adherence to best practices was regularly captured on a quarterly basis, summarized, and then sent back to hospital leaders as part of a performance feedback report.

Currently, all 16 hospitals in the regional health authority have implemented this program and 72% of the ACS population from the participating hospitals are receiving a GAP tool at discharge. Additionally, in patients who receive the GAP tool, data collection indicates an increase in adherence to all ACS best practices.

The presentation will include both pre-audit and post-implementation results, along with an overview of the barriers and challenges addressed, and how the principles of knowledge translation facilitated practice change throughout an entire health region.

**P-39**

### Smoking and Cerebrovascular disease: A Systematic Review on the Effectiveness Of Smoking Cessation Interventions (SCI) in Increasing Quitting Rates in Patients with Cerebrovascular Disease

**Rojiemiahd (RJ) Edjoc (Supervisor: Dr. Robert Reid)**

**Background:** The main objective of this systematic review is to determine the effectiveness of SCIs in increasing cessation rates in patients with established cerebrovascular disease.

Methods: Search strategy was developed with the aid of an information specialist. Searched databases included: EMBASE, MEDLINE, CENTRAL. References adjudicated by two researchers for inclusion.

**Results:** 850 relevant articles were identified from the literature. Only 4 studies fit the inclusion criteria. Given the low number of data, a meta-analysis was not possible. Cost-free pharmacotherapy and counseling may increase the likelihood of quitting, decrease cigarette consumption and reduce the absolute risk of strokes.

**Conclusions:** SCIs were found to increase cessation rates, decrease the absolute risk of strokes and reduce overall cigarette consumption. However, results from the systematic review reveal that data is still limited and a future review is suggested once more data is available.

**P-40**

### QUIT – A Pilot Trial of Standardized Counselling and Cost Free Pharmacotherapy for Smoking Cessation in Secondary Stroke Prevention

**Sophia Gocan, Smoking Cessation Specialist, Primary Care Smoking Cessation Program, Minto Prevention and Rehabilitation Center**

**Background:** Smoking cessation after stroke or TIA reduces the risk of future stroke by 50%. Baseline six-month smoke-free status in our practice setting is 13%. The absence of a coordinated approach to screening and counseling smokers, in addition to the cost of pharmacotherapy, were initially identified as potential barriers to cessation. A pilot trial was conducted to determine the feasibility and efficacy of introducing a systematic approach to cessation coupled with cost-free pharmacotherapy.

**Methods:** Beginning in August 2008, all patients at The Ottawa Hospital Stroke Prevention Clinic were screened for smoking status, and counselled and treated using a standardized protocol. Smokers willing to quit within 30 days were randomized to cost-free pharmacotherapy (CF) or prescription (P). The principal outcome was smoke free status at 26 weeks.

**Results:** 257 smokers were identified and 28 enrolled in our programme. Of the 28 participants, follow up data is available for 25 with 3/14 (21.4%) patients in the CF group and 2/11 (18.1%) in the P group biochemically validated smoke free at 6 months.

**Conclusions:** The benefits of CF pharmacotherapy accompanied with counselling demonstrate a positive trend and potential for further investigations. A larger scale trial is required to validate these observations.