Understanding the relative contributions of neoblast cells and differentiated cells to planarian regeneration

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Introduction: Fundamental knowledge on the nature of stem cell regeneration and morphogenesis is important for understanding the development and maintenance of all organisms. The planarian has emerged as a novel model to address these issues, due to its large population of stem cells, termed neoblasts, that are necessary for cell renewal and regeneration of missing tissue. Previous research using planarians has demonstrated differentiated muscle cells maintain the body pattern during regeneration by communicating with the neoblasts. This study aimed to understand neoblast and differentiated cell interactions that determine body morphology and degree of pigmentation. We hypothesized that the differentiated cells will determine the morphology and that the neoblast cells will determine the degree of pigmentation.

Methods: To examine this question, we exploited the neoblasts’ ability to restore the body of an organism that has lost its neoblasts. We attempted to restore neoblasts in one species with neoblasts from another species with a different morphology. The host species, Dugesia japonica, was irradiated to eliminate endogenous neoblasts, and then rescued with neoblasts from another species, Schmidtea mediterranea.

Results: Our preliminary results indicate that the rescue neoblasts are capable of proliferating and migrating within the host species because the neoblasts have taken proper positioning within the host. In addition, qualitatively there appears to be an increased degree of pigmentation in the host after neoblast introduction. The neoblasts and differentiated cells appear to be incapable of cell-cell communication as attempts at regeneration are unsuccessful.

Conclusions: These initial findings suggest that the degree of divergence between the two species is substantial enough to prevent effective neoblast and differentiated cell interaction.

Keywords: Neoblast cells, differentiated cells, body morphology, pigmentation, planarians
The role of aberrant Nrf2/Keap1 pathway activation across a broad spectrum of human cancers

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Abstract
Keap1 is a negative regulator of the transcription factor Nrf2, which is activated during times of oxidative stress. Keap1 mutations have been identified in non-small cell lung cancer, yet overactivation of Nrf2 and the potential role of Keap1 mutations in other cancer types have not been systemically assessed. Our goal is to investigate numerous different types of cancer for the presence of these Keap1 mutations and for the up-regulation of Nrf2. We will also investigate the molecular mechanism by which overactive Nrf2 can contribute to the cancer phenotypes. We have already explored cases of endometrial cancer, renal cell carcinoma and head and neck cancers. We tested these cases for the upregulation and nuclear localization of Nrf2 as well as localization of Keap1 using immunohistochemistry. We also use yeast models to explore the cellular mechanisms underpinning Nrf2 activation. Yeast cotransformed with the cancer-related Keap1 mutants and Nrf2 have been analyzed to give insights into toxicity associated with overactive Nrf2. We expect that the mutant Keap1 proteins will fail to interact with and de-activate Nrf2.

Keywords: cancer, oxidative stress, antioxidant, chemotherapy, reactive oxygen species, Nrf2, Keap1, immunohistochemistry, yeast model
Characterization of ALDH$^{\text{hi}}$/CD133$^+$ Cells in the Human Fetal Pancreas

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Introduction: Understanding how endocrine progenitors commit to a β-cell lineage during human fetal development is necessary for the design of in vitro β-cell differentiation protocols. Previous research has determined that high aldehyde dehydrogenase (ALDH) activity is necessary for the development and survival of β-cells. The purpose of the current study is to assess the endocrine lineage commitment of ALDH$^{\text{hi}}$/CD133$^+$ human fetal pancreatic cells and determine their potential for forming a pool of endocrine cell precursors.

Methods: Human fetal pancreata (18-22 weeks) were dissociated to a single cell suspension, labeled for ALDH and CD133, and sorted into double positive (ALDH$^{\text{hi}}$/CD133$^+$) and double negative (ALDH$^{\text{lo}}$/CD133$^-$) populations using fluorescence-activated cell sorting (FACS). Sorted populations were then cultured for an extended period to assess their phenotypic stability and then characterized using immunofluorescence staining and qRT-PCR to quantify various transcription factors and cellular markers indicative of endocrine cell commitment.

Results: Fluorescence-activated cell sorting yielded a larger population of double positive cells than double negative cells. Expression of ALDH and endocrine-specific transcription factors was not detected in either cell population after extended cell culture. Both of the expanded populations were positive for vimentin, Ki-67, CK19, SOX9 and β-catenin, with no significant differences in expression levels.

Conclusions: Our preliminary results indicate that neither population is capable of maintaining expression of ALDH or endocrine-specific markers after extended culture. Further studies should assess each population’s capacity for forming islet-like cell clusters to further characterize any underlying differences in endocrine lineage commitment.

Keywords: Aldehyde dehydrogenase, CD133, diabetes, β-cell, stem cells, FACS, human fetal pancreas
miR-200b regulates Fibronectin expression in Renal Endothelial Cells of Mice with Diabetic Nephropathy

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Introduction: Diabetes is the leading cause of end-stage renal failure in North America. Endothelial cell dysfunction is a key component in the development and progression of diabetic nephropathy. Expression of fibronectin (FN) by endothelial cells is increased in diabetes, and contributes to basement membrane thickening and mesangial matrix expansion observed in diabetic nephropathy. We have previously shown that microRNA-200b (miR-200b) plays an important role in diabetic retinopathy. Furthermore, based on bioinformatics analyses, FN is a target of miR-200b. Hence, in this study we examined whether miR-200b is important in the pathogenesis of diabetic nephropathy and whether such effects are mediated through regulation of FN production.

Methods: Transgenic mice were engineered to overexpress miR-200b under the control of an endothelial cell specific Tie2 promoter. Diabetes was induced in wild-type and transgenic C57Bl/6 mice using low dose streptozotocin. The animals were monitored with respect to body weight and blood glucose levels. Mice were sacrificed after two months of diabetes and kidneys were harvested. Tissue from the renal cortex was analysed for FN mRNA and protein expression.

Results: Diabetic mice had reduced body weight and hyperglycemia. Analyses of endothelial cells isolated from the kidneys showed significantly increased miR-200b expression in the transgenic mice compared to wild-type controls. We observed a statistically significant increase in FN mRNA in the kidneys of diabetic mice compared to wild-type controls. Both basal and diabetes-induced increases in FN were abrogated in the diabetic mice overexpressing miR-200b.

Discussion: Our data suggests that miR-200b overexpression may protect against increased extracellular matrix production in diabetic nephropathy. Future experiments will be performed to determine the mechanisms of such regulation by miR-200b.

Keywords: MicroRNA, miR-200b, fibronectin, endothelial cells, diabetic nephropathy, extracellular matrix proteins
Determining the Effect of Glucocorticosteroid Treatment on CRTH2 Expression Levels in Th2 Cells

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**Introduction:** The chemoattractant homologous receptor for Th2 cells (CRTH2) is responsible for mediating chemotaxis of inflammatory cells in an allergic response. Specifically in Th2 associated asthma, exposure to an allergen in the lung mucosa can result in increased CRTH2 activation. Glucocorticosteroid therapy is aimed to minimize the allergic response in asthmatics by reducing cytokine production and inflammatory cell recruitment. The regulation of CRTH2 expression in Th2 cells in response to glucocorticosteroid treatment is currently not well understood. This study aims to determine the effect of the glucocorticosteroid, dexamethasone, on CRTH2 expression in Th2 cells. We hypothesize that dexamethasone treatment of Th2 cells will result in increased transcription of CRTH2 in Th2 cells.

**Methods:** CCRF-CEM cells will be cultured and treated with 1-10μM of dexamethasone for 6, 24, and 48 hours to determine the effect it has on CRTH2 mRNA expression. CRTH2 mRNA transcript expressed in CCRF-CEM cells will be quantified using quantitative real time polymerase chain reaction (qRT-PCR).

**Results:** Using in silico analysis we have identified CRTH2+ and Th2 specific expression in the CCRF-CEM cell line. An in vitro assay has been developed to determine changes in CRTH2 expression in CCRF-CEM cells in response to dexamethasone treatment.

**Conclusion:** The results of this experiment will provide novel results on the effect of dexamethasone treatment on CRTH2 mRNA expression in Th2 cells. Our results may provide an incentive to further investigate a CRTH2 mediated mechanism of glucocorticoid resistance exhibited in severe asthmatic patients.

**Keywords:** Th2 Cells, CRTH2, Glucocorticosteroid, Dexamethasone, Severe Asthma, Allergic Disease
Injury Patterns Sustained in Motor Vehicle Collisions with Driver’s Third Generation Airbag Deployment

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Introduction: Following introduction of first generation airbags and consequent fatalities due to their “aggressive” nature of deployment, second and later third generation airbags were developed. While there are studies describing injuries associated with first and second generation airbags, no studies have documented injuries associated with third generation airbag deployment.

Materials: Fatal driver Motor Vehicle Collision (MVC) case files, which occurred in the year 2012, were retrieved from the Ontario Office of the Chief Coroner and reviewed. Inclusion criteria: collisions had to be full frontal or offset frontal; drivers had autopsies documenting injuries. Model of vehicle was used to determine the airbag generation which deployed during the collision. Odds ratio statistical analysis was used.

Results: In total 295 MVC were reviewed and 65 cases met the inclusion criteria of the study. Craniocerebral, cervical spinal, thoracic and abdominal injury patterns were not statistically different for third generation airbag deployments when compared to first/second airbag deployments or airbag non-deployment cases. Seatbelt use did not affect injury patterns among third generation airbag deployment collisions. When all 65 collision cases were analyzed, 17 cases sustained a combined injury pattern to thorax and abdomen, and 15 sustained a set of craniocerebral, thoracic and abdominal trauma. Most of the motor vehicle collision cases were of high impact severity (most 80 km/h and above).

Conclusions: Airbag deployment, regardless of generation, and seatbelt usage play little role in determining injury patterns in drivers killed in the collisions analyzed. High speed appears to be the critical factor in determining injury patterns.

Keywords: airbags, collisions, injuries, pathology, third generation
Expression of Growth Hormone Secretagogue Receptor 1a and Ghrelin in Human Heart Failure

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Introduction: Currently the prognosis for heart failure (HF) remains poor and there is no single diagnostic test for HF. The development of new cardiac-specific biomarkers for HF could greatly improve HF diagnosis. Our study aimed to investigate whether myocardial levels of growth hormone secretagogue receptor 1a (GHS-R1a) could potentially serve as a biomarker for HF.

Methods: A previously characterized ghrelin analog, Cy5-ghrelin (1-19), was used to assess GHS-R1a levels in the right atrium (RA) of explanted and implanted hearts of 2 transplant patients, and of samples from 5 cardiac surgery patients with varying severities of HF. Ghrelin levels in the RA of transplant and surgery patients were also assessed using fluorescence immunohistochemistry.

Results: Levels of both GHS-R1a and ghrelin were significantly increased in diseased explanted hearts as compared to healthy implanted hearts of transplant patients (p ≤ 0.0001). Levels of GHS-R1a were significantly increased in 3 surgery patients in comparison to healthy implanted hearts of transplant patients (p ≤ 0.01). Levels of ghrelin were significantly increased in all 5 surgery patients in comparison to healthy implanted hearts of transplant patients (p ≤ 0.001).

Discussion: Our study represents the first quantitative findings of significantly increased GHS-R1a and ghrelin levels in the RA of explanted hearts as compared to healthy implanted hearts of transplant patients. In contrast to our initial hypothesis, these findings suggest a parallel increase in both GHS-R1a and ghrelin levels in HF. Our study encourages further research aimed at understanding ghrelin system changes in HF and future potential use of GHS-R1a as a biomarker for HF.

Keywords: Heart failure, biomarkers, ghrelin, growth hormone secretagogue receptor, GHS-R1a, Cy5 ghrelin (1-19), fluorescence
Growth Differentiation Factor 15 has a Protective Role in LPS-induced Acute Kidney Injury

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Introduction: Septic acute kidney injury (AKI) is a leading cause of morbidity and mortality with no accepted method of therapy. Growth differentiation factor 15 (GDF15) is induced in many diseases, however its role in Lipopolysaccharide (LPS)-induced AKI is unknown. We aim to determine the role of GDF15 in LPS-induced AKI to assess its therapeutic potential. As GDF15 is known to have anti-inflammatory and anti-apoptotic effects, we hypothesize that GDF15 has a protective effect in LPS-induced AKI in the mouse model.

Methods: GDF15 knockout (KO), transgenic (TG) and wild-type (WT) mice received 4mg/kg injections of LPS. Renal function was determined using serum BUN and creatinine levels. Kidney tissues were stained with H&E and scored based on area of tubular damage. Apoptosis and neutrophil infiltration were also detected by the TUNEL and MPO assays, respectively. The expression of inflammatory mediators was measured by quantitative RT-PCR.

Results: LPS treatment induced AKI as evidenced by the increased levels of BUN and serum creatinine. Our results show GDF15 KO mice treated with LPS had the highest levels of BUN and creatinine. In contrast, GDF15 TG mice had significantly lower BUN and creatinine levels compared to KO, indicating that GDF15 has a protective effect in LPS-induced renal injury. GDF15 KO mice generally had greater levels of tubular damage, as indicated by swollen epithelia and luminal precipitate in histological sections, compared to GDF15 TG mice. We also found that GDF15 deficiency in KO mice augmented the expression of TNF-α and MCP-1 compared to WT mice.

Conclusion: Our results show that GDF15 may play a protective role in LPS-induced acute kidney injury, potentially through inhibiting the NFκB pathway. We continue to elucidate the underlying mechanism by examining other inflammatory mediators at both the mRNA and protein level and through in-vitro studies.

Keywords: Septic acute kidney injury, LPS, nephrotoxin, GDF-15, NAG-1, inflammation
Cation-Permeable Channel TRPM7 is found in the Ganglion Cell Layer of the Mouse Retina

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**Introduction:** Recent evidence has indicated that ischemia may play a role in Retinal Ganglion Cell (RGC) death that is characteristic of open-angle glaucoma. Ischemia can be brought on by a variety of factors such as ocular hypertension which is found in 50% of glaucoma presentations, as well as other pre-disease states. Transient receptor potential melastatin member 7 (TRPM7) is a non-selective cation-permeable channel that conducts ions such as Ca\(^2+\) and Mg\(^2+\) across the plasma membrane, and has been implicated in neuronal death following transient ischemia-reperfusion. TRPM7 is up-regulated shortly after hippocampal ischemia-reperfusion, and conducts a large positive current into the cell in ischemic conditions. This study will investigate the role of TRPM7 in the retina. We hypothesize that TRPM7 is expressed in RGCs and plays a role in cell death following ischemia-reperfusion.

**Methods:** This study employed immunofluorescence on sagittal sections of young adult mouse eyes to localize TRPM7 to retinal cell layers with particular focus on the ganglion cell layer. We then harvested retinas and cultured primary mixed retinal cells for fluorescent immunocytochemistry and confocal analysis of TRPM7 expression in particular cell types.

**Results:** Our immunofluorescence studies on sagittal eye sections of wild-type mice show that TRPM7 is expressed in the retina. TRPM7 localizes to the ganglion cell layer, as well as the inner and outer plexiform layers of the retina. Confocal analysis of primary retinal cultures shows that ganglion cells are present in the cell culture.

**Conclusions:** These preliminary findings pave the way for further studies which will investigate whether TRPM7 plays a role in ganglion cell death. Future experiments will test the effects of acute and chronic oxidative stress on ganglion cell death, and examine whether TRPM7 inhibitors increase cell survival under these conditions.

**Keywords:** Retina, glaucoma, ganglion cells, TRPM7, ischemia
Implementation of the serum/plasma methylmalonic acid test at the London Health Sciences Centre

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Introduction: Methylmalonic acid (MMA) is the free acid form of methylmalonyl-CoA, an intermediate in the conversion of the breakdown products of various macromolecules to succinyl-CoA, which enters the Krebs cycle. Levels of methylmalonic acid in serum or plasma can be measured, and are used in the diagnosis of diseases such as vitamin B12 deficiency and congenital methylmalonic acidemia. In this study, we attempted to implement the serum/plasma MMA test by gas-chromatography/mass spectrometry (GC/MS) in the Toxicology/TDM Lab at LHSC.

Methods: The mass spectra of MMA and its trideuterated analogue were obtained using a standard scan program in the GC/MS instrument. A literature-based method was then programmed into the instrument, and the initial sample preparation steps were modified to optimize the sensitivity and peak shapes of the analytes. Horse serum-based standards were used to establish a calibration curve that was used in the calculation of MMA levels in patient samples.

Results: The calibration curve was linear, and the endogenous level of MMA in the horse serum was calculated to be approximately 400nmol/L. The upper quality control samples (550nmol/L) exhibited acceptable values of within-day and total coefficient of variation that were under 15%, while the lower quality control samples (320nmol/L) showed more variability. The MMA levels in 10 of 12 randomly selected patient samples tested were in the reference range (100-400nmol/L), with two samples somewhat higher.

Conclusions: The complexity and difficulty associated with the setup of the test were demonstrated by various problems with both the instrument and the sample preparation methods – both the GC/MS settings and the initial preparation procedure were altered during the duration of the project. We did not reach the point where we could perform correlation experiments with patient samples that had been previously sent to the reference lab for analysis, and the test will have to be further developed.

Keywords: Methylmalonic acid, vitamin B12 deficiency, congenital methylmalonic acidemia, gas chromatography, mass spectrometry, isotope dilution
Targeting T memory cells with Receptor Interacting Protein Kinase 3 (RIPK3) mediated Necroptosis

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Introduction: Post-operative graft rejection is a risk that cardiac transplant patients live with for the rest of their lives. Hence, post-operative drug regimens include immunosuppressive drugs to prevent allograft rejections. The use of immunosuppressive drugs also poses a risk for the patient in terms of immunity. The current goal is to find a novel immunomodulatory strategy, specifically by inducing cell death of T memory cells involved in graft rejection to prevent rejections. The key player in graft rejections are T memory cells involved in the immune response against the foreign organ. Inducing T memory cell death remains elusive, due to the anti-apoptotic quality of T memory cells. We hypothesize that the RIPK3-mediated necroptotic pathway can be used to induce T cell death.

Methods: CD4+ T cells and CD8+ T cells of the Balb/C mouse background will be cultured from the spleen and lymph node and activated by mixed lymphocyte reaction (MLR) using B6 splenocytes. After 5 days, we will induce TNF-mediated necroptosis. The level of death and type of the death will be measured using flow cytometry, immunohistochemical staining, and immunoblot for levels of high mobility group box 1 protein (HMGB1). We will repeat this using T memory cells.

Results: Our results show that TNF-mediated necroptosis can be induced in CD4+ and CD8+ T cells by using TNFα, SMAC mimetics and Z-VAD-FMK and reduced by using Necrostatin-1 to inhibit RIPK3.

Conclusion: The findings show that the death of CD4+ and CD8+ T cells can be manipulated. The results provide us with the conditions required to induce necroptosis in T cells.

Keywords: Graft rejections, T memory cells, apoptosis, necroptosis, necrosis, RIPK3, TNFα-mediated cell death, cardiac transplants
Effect of Mechanical Stretch on Extracellular Matrix of Human Trabecular Meshwork Cells

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Introduction: Open-angle glaucoma is a result of excess accumulation of extracellular matrix (ECM) within the trabecular meshwork (TM). This prevents proper outflow of the aqueous humor, resulting in elevation of intraocular pressure (IOP) and optic nerve damage. It is known that phosphatase and tensin homolog (PTEN) is a major regulator of ECM remodelling, and increased mechanical strain on the TM induces deposition of excess ECM. Since IOP elevation resulting from glaucoma increases mechanical stretch on the TM, we have investigated the changes in expression of PTEN as well as changes in ECM with application of physiological and pathological mechanical stretch on human TM cells. We hypothesize that mechanical stretch on human TM cells modulates expression of PTEN. This regulation of PTEN in turn modulates the deposition or degradation of ECM.

Methods: Human TM cells from embryonic donors were subjected to physiological (5%) and pathological (15%) mechanical stretch using the FX5000 Tension System at 1Hz for 24 and 48 hours. Proteins were then extracted and analyzed for the expression of PTEN and collagen using immunoblot.

Results: Our results show that there is a significant elevation in the expression of type I collagen by TM cells as mechanical stretch is increased in duration (24 to 48 hours) and magnitude (5% to 15%). At 15% stretch for 48 hours, expression of PTEN decreased, and this was associated with higher levels of collagen in ECM.

Discussion: Our results indicate that pathologic stretch of TM, as in patients with glaucoma, decreased the expression of PTEN and thus could be a factor in the accumulation of excess ECM in TM. This finding signifies that increasing the activity of PTEN could be a valuable therapeutic target to treat glaucoma by decreasing ECM deposition in TM and thus reducing IOP.

Keywords: glaucoma, mechanical stretch, trabecular meshwork, intraocular pressure, PTEN
Upregulation of ATP Synthase 5A1 ameliorates calpain-mediated mitochondrial ROS generation and diabetic cardiomyopathy

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Introduction: Cardiomyopathy is arguably the most concerning complication of diabetes. It is characterized by the apoptosis, hypertrophy, and fibrosis of cardiac muscle. The protease calpain is an important mediator of diabetic cardiomyopathy. Our lab has previously shown that cardiomyocytes isolated from diabetic mice display increased calpain activity. The current study aims to investigate a specific pathway by which calpain inhibits the ATP synthase 5A1 subunit in the mitochondria to generate reactive oxygen species (ROS) and induce cardiomyopathy. As well, the potential for ATP synthase 5A1 upregulation to serve as a therapy will be investigated.

Methods: A diabetic mouse model was created using streptozotocin (STZ), and the heart was surgically isolated once diabetes was successfully induced. Calpain activity in the mitochondria was measured using the fluorescent substrate N-succinyl-LLVY-AMC, and ROS level in the mitochondria was measured using Amplex® Ultrared. Interaction between calpain and ATP synthase 5A1 was shown using immunoprecipitation. Upregulation of ATP synthase 5A1 was achieved using adenovirus transfection. Finally, fluorescence microscopy and the ImageJ software was used to measure the degree of cardiomyopathy, based on the extent of hypertrophy and fibrosis.

Results: Our results show that calpain activity and ROS level are both elevated in the mitochondria of diabetic cardiomyocytes. This is mediated through ATP synthase 5A1 inhibition, since overexpression of the ATP synthase 5A1 gene resulted in decreased ROS generation, hypertrophy, and fibrosis. This is further confirmed by immunoprecipitation results showing interaction between calpain and ATP synthase 5A1.

Conclusions: Our findings suggest that diabetes results in increased mitochondrial calpain activity and inhibition of ATP synthase 5A1 subunit, ultimately leading to diabetic cardiomyopathy. These findings reveal a specific pathway of the disease and propose a potential therapy.

Keywords: diabetes, cardiomyopathy, calpain, ATP synthase 5A1, cardiomyocytes, ROS
Effects of the Western diet and IUGR on Glucose Homeostasis

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Introduction: Intrauterine growth restriction (IUGR) induced by uteroplacental insufficiency has been shown to predispose individuals to visceral obesity and type 2 diabetes. Similarly, a diet high in saturated fat and fructose, characteristic of the Western diet, has been linked to the development of obesity and type 2 diabetes. Previous studies done in humans and rodents, indicate that both can increase the risk of altered lipid homeostasis, insulin resistance, and β-cell dysfunction in late adulthood. The current study will examine the changes in the morphology of the endocrine pancreas by the single or combined treatment. We hypothesize that a Western diet in combination to placental insufficiency will further alter pancreatic development and predispose individuals to glucose intolerance earlier in life.

Methods: Guinea pigs were used due to their development of a more mature pancreas at birth. Normal birth weight and low birth weight (induced by uterine artery ablation) pups were either fed a control diet or a Western diet until sacrifice. Pancreata were collected at postnatal day 145 and tissues were examined by single or dual immunohistochemistry to detect glucagon and insulin. Microphotographs were taken to determine the number of islets, size of islets, α-cell area, β-cell area, and total islet area.

Results: The results will show that the presence of IUGR and a Western diet, individually and in combination, will cause a decrease in the number of islets, α-cell area, β-cell area, and total islet area as well as a differing distribution of islet size in comparison to their respective controls.

Conclusions: These findings show that dietary exposure affects the morphology of the endocrine pancreas, that IUGR may affect the pancreas development in utero and this may affect islet morphometry later in life and that the combined effect of a Western diet and IUGR will increase the risk of β-cell failure earlier in life.

Keywords: Uteroplacental insufficiency, intrauterine growth restriction, Western diet, β-cell, type 2 diabetes