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The effect of maternal obesity of developmental gene expression in the fetal arcuate nucleus. H Twigg, C Jasoni. Centre for Neuroendocrinology and Department of Anatomy, Otago School of Medical Sciences, University of Otago, Dunedin.

It has been well established that changes in the uterine environment can affect fetal development. Additionally, it has been further shown that maternal obesity is associated with increased risk of the development of obesity in the offspring, however the mechanism of this relationship is not fully understood. We, and others, have observed that the offspring of obese mothers show altered development of axonal projections from cells in the hypothalamic arcuate nucleus (ARC), known to be one of the key brain areas for body weight regulation. Based on this, we hypothesised that the expression of genes that regulate axon growth and guidance would be altered in fetuses developing in obese mothers when compared with mothers of normal weight.

Quantitative-RT-PCR (qRT-PCR) was used to assess gene expression in the mouse ARC at gestational day 15.5 (GD15.5), representing mid-gestation, and early arcuate development. Specifically, we examined the *Robo* genes (*Robo1*, *Robo2* and *Robo3*), and the *Slit* family (*Slit1* and *Slit2*), which produce ligands for Robo receptors, and are involved in directing axon guidance.

Robo2 expression is significantly up-regulated (1.96-fold ± 0.37 (SEM) $P < 0.05$, Student's t test) in offspring from mothers on a high fat diet (MHF) compared with controls. *Robo3* expression is significantly down-regulated (0.53-fold ± 0.18 (SEM) $P < 0.05$) in MHF females compared to controls. *Slit2* expression is up-regulated (1.38-fold ± 0.07 (SEM) $P < 0.01$, Student's t test) in MHF males, and this change in expression is highly significant.

The results presented in this study indicate that changes in *Robo* and *Slit* expression could provide a molecular mechanism behind malformation of weight regulation circuitry in offspring from obese mothers. With the incidence of obesity in the developed and developing world reaching epidemic proportions, an understanding of the mechanisms that underpin elevated risk for obesity are critical in order to best confront this disease.

Increased sympathetic activity in obesity may protect against pulmonary hypertension. C Diong, E Gray, P Jones, D Schwenke. Department of Physiology, Otago School of Medical Sciences, University of Otago, Dunedin.

Evidence suggests that obesity is protective against pulmonary hypertension (PH) which is a disease characterised by an elevation of mean pulmonary arterial pressure (MPAP). It is well established that pulmonary sympathetic nerve activity (pSNA) is

an important modulator of MPAP due to ß-adrenergic-mediated vasodilation of pulmonary vessels. This study aimed to establish whether pSNA is elevated in obesity and how this impacts vascular tone.

Using *in vivo* electrophysiology, we directly recorded pSNA in anaesthetised Zucker rats and showed that pSNA in control obese rats (obese-C; 2.4 ± 0.4 µV.s; mean ± S.E.M; n = 8) was significantly elevated compared to their lean controls (lean-C; 0.5 ± 0.1 µV.s; n = 7; $P < 0.001$; unpaired *t*-test). PH led to a small 2.9-fold increase in pSNA in lean rats (lean-PH; 2.0 ± 2.5 µV.s; n = 4) but a large 13.2-fold increase in the obese rats (obese-PH; 7.1 ± 2.5 µV.s; $P < 0.01$; n = 4).

To determine the effect of differing pSNA on vessel diameter, we performed synchrotron radiation microangiography. We blocked the effect of pSNA by administrating the ß-adrenergic antagonist propranolol (2 mg/kg) and then induced acute hypoxic pulmonary vasoconstriction using 8% O_2 for 5 minutes. The magnitude of constriction in lean-C and lean-PH rats was 12.6 ± 3.5% (n = 7) and 16.3 ± 3.4% (n = 7), respectively. However, compared to lean-C rats, pSNA blockade in obese-C (26.2 ± 3.7%; n = 7; $P < 0.05$) and obese-PH rats (27.6 ± 4.7%; n = 7; $P < 0.05$) resulted in a significantly greater magnitude of vasoconstriction.

Our results suggest that pSNA is significantly elevated in obesity and plays a larger role in vasodilation of the pulmonary vasculature. This may in part explain why obesity in PH results in an improved clinical outcome.

Phenotypic and functional characterisation of human macrophages – a role in colorectal cancer. **S Norton[1], E Dunn[1], F Munro[2], J McCall[2], R Kemp[1]. [1]Department of Microbiology and Immunology, Otago School of Medical Sciences. [2]Department of Surgical Sciences, Dunedin School of Medicine, University of Otago, Dunedin**

In contrast to other cancers, high infiltration of macrophages into the tumour of people with colorectal cancer has been associated with improved patient prognosis. This association may be due to limitations of markers used to identify macrophages *ex vivo*. Thus, the mechanisms involved in macrophage responses to cancer are poorly understood. Our goal was to establish macrophage subset identification methods and analyse whether these subsets infiltrate colorectal tumours.

Multicolour flow cytometry was used to identify macrophage populations derived from blood, and differences in function confirmed using ELISA, Greiss reaction and quantitative RT-PCR.

M1 macrophages were identified as CD11b+, CD64+, CD14-, CD206+/lo, CD163-; and highly expressed the nitric oxide synthase 2 gene, indicating pro-inflammatory function. M2 populations were CD11b+, CD64+, CD14hi, CD206+, CD163+, and produced high levels of interleukin-10 protein, indicating anti-inflammatory function. These combinations of surface markers represent a novel way to measure macrophage subsets *ex vivo*. These markers are often used to identify macrophages, but not in combination. CD206 was chosen as an M2 marker, but was highly expressed on M1 macrophages. The two populations were subsequently detected in tumour and associated non-transformed bowel tissue from three people with colorectal cancer

(M1 = 1.12 ± 1.07, M2 = 0.637 ± 0.090 and M1 = 4.45 ± 3.57, M2 = 0.129 ± 0.066, mean % immune cells ± SEM, respectively). There appears to be an increased frequency of M2 macrophages in tumour tissue. Analysis revealed a population of gut resident macrophages (9.58 ± 2.92 and 11.5 ± 4.179) with a unique phenotype (CD45+, CD11b-, CD64-, CD14-, CD206+ CD163-), present in both tumour and non-transformed bowel tissue.

This study highlights the complexity of macrophages and provides methods to examine them *ex vivo* from human tissue. Data gained from this work may help define macrophage type as either prognostic or therapeutic targets.

Secreted amyloid precursor protein-α attenuates cell death in organotypic hippocampal slices. **M Elder[1], J Blok[1], B Mockett[2], J Williams[1]. Brain Health Research Centre, [1]Department of Anatomy, Otago School of Medical Sciences, [2]Department of Psychology, University of Otago, Dunedin.**

Alzheimer's disease (AD) is a neurodegenerative condition characterized by deposition of amyloid beta (Aβ) and apoptotic cell death, especially in the hippocampus. Curiously, neurotoxic Aβ is cleaved from amyloid precursor protein while alternate cleavage liberates the neuroprotective molecule, secreted amyloid precursor protein-α (sAPPα). This study aimed to investigate whether sAPPα application could prevent cell death in organotypic hippocampal slices following Aβ insult or the generic cell death-inducing oxygen glucose deprivation (OGD).

Hippocampal slices (400 μm) from P7-10 Sprague-Dawley rat pups were plated on membranes and maintained at 34-37°C for 10 days. Slices were treated with either 1 nM sAPPα or phosphate buffered saline prior to insult (either 20 minutes of exposure to an anoxic and glucose-free environment (OGD, $n = 6$) or Aβ 25-35; 2.5 μM, 48 hours ($n = 4$). Activated caspase-3 levels were used as a measure of apoptosis and assessed by western blot analysis and LIVE cell staining. Cell death was measured by incubation with propidium iodide (PI) (7.5 μM, 24 hours) and visualized by fluorescence microscopy.

While OGD produced robust cell death in hippocampal areas CA1 and CA3, preincubation with sAPPα showed no neuroprotective effect as assessed by PI ($P \leq 0.05$, one-way ANOVA). However, western blot analysis revealed significantly decreased activated caspase-3 levels following sAPPα application compared to untreated OGD slices ($P < 0.05$, one-sample t-tests). LIVE cell imaging corroborated the presence of apoptosis following OGD. Furthermore, pretreatment with sAPPα significantly attenuated cell death in the dentate gyrus following Aβ insult ($P < 0.05$, one-way ANOVA with Tukey's post hoc).

In conclusion, sAPPα pre-treatment was insufficient to prevent death following OGD however reduced apoptosis was observed following OGD and Aβ insult. Together these findings may assist in the pursuit of a treatment to prevent the loss of hippocampal neurons in AD.

Prefrontal cortex stroke affects learning and memory. L Zhou[1], A Clarkson[1,2]. [1]Department of Anatomy, Otago School of Medical Sciences, [2]Department of Psychology, University of Otago, Dunedin.

Stroke is a leading cause of long-term disability and can affect motor, sensory, language, vision and cognition (memory, attention and executive function). Research into motor impairments is well established, however little is known about the effects on cognitive deficits after stroke. Therefore we aimed to establish a stroke model that would allow us to assess components of learning and memory. We have chosen to target the prefrontal cortex (PFC), as epidemiological evidence has shown that small strokes to PFC areas are linked to cognitive impairment.

Strokes were induced in 3-month old C57Bl/6J male mice using either the photothrombotic (PT) model of stroke with 18, 20 or 22-minutes light exposure (n = 2, 4, 5 respectively) or an injection of N5-(1-iminoethyl)-L-ornithine, dihydrochloride (n = 2). Using the 22-minute PT model of stroke, mice received either sham (n=9) or stroke (n = 10) surgeries and assessed on novel object (NO) and the object-location recognition (OLR) tasks. Open field and elevated plus maze (EPM) tests were used to monitor motor and anxiety levels in mice.

Cresyl-violet staining and ImageJ software were used to quantify the area of infarction. These infarct quantifications show that 22-minute PT stroke was the most consistent size and reliable. For both the open field and EPM, no significant differences were found between sham and stroke mice at 1 and 4-weeks post stroke ($P \geq 0.05$). Assessment on the NO task also showed no differences at 1 and 4-weeks between sham and stroke mice ($P \geq 0.05$). Assessment however on the OLR task stroke mice were found to have no impairment at 1-week, but significant impairment at 4-weeks post-stroke compared to sham mice ($P \leq 0.05$).

This is the first experimental evidence that strokes to the PFC result in a delayed onset impairment in the OLR task, similar to human studies. We suggest that this model may therefore be a useful tool in assessing potential rehabilitative/cognitive therapies after stroke.

Inhibitory role of Langerhans cells (LCs) during cutaneous wound healing. J Ann, L Wise, N Real, G Stuart, M Hibma. Department of Microbiology and Immunology, Otago School of Medical Sciences, University of Otago, Dunedin.

Langerhans cells (LCs) are epidermal dendritic cells (DCs) that play a pivotal role in skin immunity as well as maintaining tolerance. Previous research has suggested LCs may also be involved in the wound healing response, as changes in LC number have been observed following cutaneous injury and in injury-associated skin diseases. The aim of this study was to determine if LCs play a regulatory role during skin repair.

Mice expressing the diphtheria toxin (DT) receptor from the langerin/CD207 promoter were treated with DT (two intraperitoneal injections of 1 µg/kg) so as to selectively deplete LCs. Mice then received full thickness, cutaneous, punch (4 mm) wounds above each hind-limb. Six days post-wounding, mice were euthanised and wounds were fixed in zinc-salts solution, paraffin-embedded then (4 µm) sections

were stained with Martius Scarlet Blue (MSB) or by immunofluorescent-histochemistry.

Staining for langerin/CD207 confirmed that LCs were depleted in DT-treated mice. Depletion of langerin/CD207$^+$ cells resulted in an immediate 30% reduction in wound size compared to controls (day 1-2 post-wounding, n = 8, $P < 0.05$, ANOVA/Bonferroni's *post hoc* test). MSB-stained sections revealed increases in the area of neo-epidermis (30%, n = 8, $P < 0.05$, unpaired t-test) and granulation tissue (70%, n = 8, $P < 0.05$) in LC-depleted wounds compared to controls. Fluorescent-stained sections revealed a 2.5-fold reduction in MHC class II$^+$ dermal DCs (n = 8, $P < 0.05$) and increases in F4/80$^+$ macrophages and CD8$^+$ T cells (n = 2) adjacent to the granulation tissue of LC-depleted wounds compared to control wounds.

In conclusion, LCs may inhibit the inflammatory and proliferative phases of cutaneous wound healing by modulating trafficking of dermal DCs, macrophages and CD8$^+$ T cells. Understanding the complex dynamics of the immune system during skin repair may lead to improved therapies for skin wounds and their associated diseases.

The effect of riboceine on glutathione and plasma cholesterol levels in Lp(a) mice.
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Elevated concentrations of lipoprotein(a) [Lp(a)] are an independent risk factor for developing atherosclerosis. Lp(a) accumulates oxidised phospholipids (OxPL) and promotes lipid deposition and inflammation in the artery. To date, there is no effective therapy available to reduce these atherogenic properties of Lp(a). Riboceine is a cysteine analogue designed to increase anti-oxidant glutathione (GSH) synthesis. As GSH is an essential cofactor for the reduction of OxPL, we hypothesized that increased GSH levels may reduce the OxPL content of Lp(a) and thereby reduce its atherogenicity. As the pathways of cholesterol synthesis or efflux are regulated by OxPL, we hypothesized riboceine may alter total plasma cholesterol levels.

Lp(a) mice were supplemented with riboceine (4 mg per day) in their drinking water for 8 weeks. High Performance Liquid Chromatography (HPLC) was used to measure GSH quantitatively in the plasma and liver tissue of the Lp(a) mice and total cholesterol levels were measured by enzymatic assay.

There was a trend towards increased GSH levels in the plasma of riboceine-treated mice compared to controls (2.13 ± 0.54 nmol/mL (mean ± SEM) versus 1.31 ± 0.17 nmol/mL, respectively) but not to statistical significance ($P = 0.1914$, n = 4, two-tailed unpaired student t-test). There was also a trend for increased GSH in the livers of treated animals (0.92 ± 0.08 µmol/g versus 0.72 ± 0.06 µmol/g, respectively; $P = 0.0821$, n = 4, two-tailed unpaired student t-test). Total cholesterol levels were 79.49 ± 6.47 mg/dL and 57.45 ± 5.91 mg/dL before and after the treatment, respectively, although this difference was not significant ($P = 0.1050$, n = 4, two-tailed paired student t-test).

These results suggest that riboceine may increase GSH levels which could alter Lp(a) atherogenicity as well as reduce cholesterol levels. However, more animals need to be treated to establish if these trends become significant.

Regulation of voltage-gated calcium channels in PC12 cells by Leucine Rich Repeat Kinase 2. C Bedford, S Condliffe. Department of Physiology, Otago School of Medical Sciences, University of Otago, Dunedin.

Leucine rich repeat kinase two (LRRK2) is a widely expressed protein belonging to the Roco family of proteins, mutations in which have recently been discovered as a cause of familial Parkinson's disease (PD). Despite an array of interacting proteins having been identified across multiple cellular systems, LRRK2's functional role remains to be determined. Manipulation of LRRK2 expression disrupts many Ca^{2+}-dependent cellular processes. It therefore may act as an upstream regulator of initial Ca^{2+} signaling events which could explain LRRK2's widespread effects.

The central aim of this study was to determine whether LRRK2 alters endogenous voltage-gated Ca^{2+} (Ca_V) channel function in PC12 cells using whole cell patch clamp electrophysiology. Additionally, transiently transfected PC12 cells underwent epifluorescence imaging to identify morphological changes and identify any effects of L-type Ca^{2+} blockers on neurite morphology.

Peak Ca_V channel currents in LRRK2 transfected cells showed a significantly (P = 0.0025, $n \geq 7$, one way ANOVA with Tukeys *post-hoc* test) higher current density across a number of holding voltages relative to untransfected and EGFP-transfected controls. This results indicates that LRRK2 up regulates endogenous Ca_V channel function. Morphological assessment showed no significant effect of LRRK2 transfection on total neurite length relative to EGFP transfected and non-transfected controls ($P>0.05$, $N\geq63$, Kruskal-Wallis test). Furthermore, addition of a L-type Ca^{2+} channel blocker Nifedipine (1 µg/mL) had no significant effect on total neurite length relative to untransfected controls ($P > 0.05$, $n \geq 43$, Kruskal-Wallis test). These results suggest that LRRK2 dependent modulation of Ca_V channel function does not affect neurite differentiation.

Overall, this study has identified a novel effect of LRRK2 on Ca_V channels which may explain how LRRK2 has such widespread cellular effects, and advances our understanding of LRRK2s functional role. If the effect of LRRK2 on Ca_v channels is responsible for pathology, Ca_v channel blockers currently being investigated for Parkinson's therapy, may be important for Parkinson's patients harbouring LRRK2 mutations.