Dynamics of myogenic progenitors in the rat hindlimb – a role for innervation. B Hurren, M Duxson. Department of Anatomy, Otago School of Medical Sciences, University of Otago, Dunedin.

Development of a functional relationship between nerve and muscle is important both embryonically and throughout postnatal life. A breakdown of this relationship can lead to diseases such as motor neuron disease and muscular dystrophies. In order to better understand the relationship between developing muscle and nerve, we used the rat as an animal model to investigate the effect of a chemical denervation in utero on the development of muscle progenitor cells expressing the paired homeobox genes Pax3 and Pax7 - which are early markers of the limb myogenic lineage.

In normal embryos, immunohistochemical examination showed that Pax3\(^{+ve}\) progenitors were first seen in the hindlimb at embryonic day (E) 12.5, followed by Pax7\(^{+ve}\) progenitors one full day later at E13.5. The nerve entered the limb at the same time and in close proximity to the first Pax7\(^{+ve}\) progenitors. Denervation in utero by injection of beta-bungarotoxin (BTX) into the embryonic peritoneum at either E15.5 or E16.5, followed by immunohistochemical examination of extensor digitorum longus (EDL) and tibialis anterior (TA) muscles either 24 h or 48 h later, revealed a decrease in the number of Pax7\(^{+ve}\) progenitors compared to controls (e.g. at 48 h: control TA / EDL: 248.8 ± 5.3 / 123.7 ± 3.6, BTX TA / EDL: 218.5 ± 3.4 / 85.1 ± 2.2, \(P < 0.0001\), \(n = 6\), Student’s t-test). Concurrently, after denervation we saw an increase in the number of cells expressing myogenin (a marker of muscle differentiation) and increased apoptosis of Pax7\(^{+ve}\) progenitors (assessed by active caspase-3 labeling). Quantitative PCR analysis corroborated these findings, with many genes associated with differentiation and apoptosis being upregulated, and genes associated with proliferation and cell cycle regulation being downregulated after denervation.

From these results, we conclude that differentiation and survival of Pax7\(^{+ve}\) myogenic progenitors is critically dependent on developmental interactions with the muscle nerve.
Immunisation using a sustained release vaccine generates CD8 T cell memory population in the gut. A Highton¹, A Girardin¹, S Hook², R Kemp¹. ¹Department of Microbiology and Immunology, Otago School of Medical Sciences, ²School of Pharmacy, University of Otago, Dunedin.

Understanding how to generate an effective memory population of quantity, quality and in the correct biological location is key in having a good vaccination method. Further, it is apparent that, although humoral immune responses conferred through current vaccination methods are effective, in many cases there is still a need for vaccination that will confer a cytotoxic CD8 T cell response. We investigated the generation of murine CD8 memory T cells using a sustained antigen release vaccine vehicle (chitosan gel) and conventional dendritic cell (DC) vaccination. The aims of this work were to evaluate the efficacy of sustained release vaccines and to compare their ability to generate peripheral versus gut associated CD8 T cell memory.

Mice were vaccinated subcutaneously with chitosan gel ± ovalbumin or DCs pulsed with ovalbumin. After 30 or 60 days mice were euthanised and cells harvested from peripheral or gut associated lymphoid tissues were phenotyped using flow cytometry and assessed for functional cytotoxicity. We found higher numbers of CD8 memory T cells specific for ovalbumin in both peripheral and gut associated lymphoid tissues after vaccination with chitosan gel compared to DC vaccination (mean ± SEM, 38827 ± 6260 and 10416 ± 2178 cells, respectively, n = 3). Vaccination with chitosan gel, but not DCs, showed a cytotoxic response in vivo up to 60 days. Interleukin-7 receptor expression, important for memory cell survival, differed between peripheral and gut associated memory T cells after chitosan gel vaccination (median fluorescence intensity ± SEM, 172 ± 8 and 221± 33 respectively, n = 3).

These results indicate that subcutaneous vaccination using chitosan gel can generate a population of functional CD8 memory T cells in gut associated lymphoid. This form of vaccination could be an easier way to induce immunity in sites that are not easily accessible such as the gut.

Understanding the mechanisms behind levodopa-induced dyskinesias. L Smith¹, E Duncan², L Parr-Brownlie¹, M Black², P Dearden², J Reynolds¹. ¹Department of Anatomy and the Brain Health Research Centre, ²Department of Biochemistry, University of Otago, Dunedin.

Levodopa is the gold standard pharmacotherapy for Parkinson's disease. However with prolonged use abnormal involuntary movements known as levodopa-induced dyskinesias (LIDs) develop in up to 80% of patients. The mechanism underlying LIDs and why some patients do not develop them is unknown. The aim of this project was to determine the gene expression profile associated with the presence of LIDs using a rodent model of Parkinson's disease.

Adult male Wistar rats received an intracerebral injection of the neurotoxin 6-hydroxydopamine to lesion the dopamine system unilaterally. Successful lesioning was verified using behavioural tests, and confirmed post-mortem using immunohistochemistry. Two weeks post-surgery, rats received treatment with levodopa (L-DOPA methyl ester; 3-4 mg/kg s.c.) and benserazide (7.5 mg/kg s.c.) or benserazide only (control) twice daily for three weeks. This regimen induced dyskinesias in half the levodopa treated rats, determined by a rodent scale of abnormal involuntary movements. Following levodopa dosing, tissue
was sampled from the lesioned striatum and RNA extracted for next-generation sequencing. To minimise biological variation, RNA was examined in two pools of three rats per condition (dyskinetic, non-dyskinetic, control; n = 6 per condition). Gene expression in these samples was measured using RNA-seq on the Illumina HiSeq 2000. Sequences were mapped to the rat genome with annotation RGSCv3.4.65. Differential expression analysis was performed using Baggerly’s test, with a false discovery rate threshold of 0.05 used to determine significance.

One hundred and fifty-nine genes were significantly differentially expressed between dyskinetic and non-dyskinetic rats; ninety-seven genes between dyskinetic and control rats. Gene Ontology analysis using Ingenuity Pathway Analysis software revealed changes related to GABA receptor signalling and calcium signalling. Overall, these results show that LIDs alter many aspects of neuronal signalling and function, which may underlie motor effects observed in Parkinson’s patients.

Orthopaedic biomaterials: Does magnesium have a promising future? S Shadanbaz¹, J Walker¹, M Staiger², T Woodfield³, G Dias¹. ¹Department of Anatomy, University of Otago, Dunedin. ²Department of Mechanical Engineering, University of Canterbury, Christchurch. ³Department of Orthopaedic Surgery and Musculoskeletal Medicine, University of Otago, Christchurch.

Magnesium (Mg) is a lightweight metal with degradable properties that has been suggested as a revolutionary replacement of current inert metallic materials. Advantages include osteogenic properties, biocompatibility and an elastic modulus comparable to human bone. However, if the corrosive nature of Mg is not controlled, premature mechanical failure and/or excess hydrogen production with resultant tissue damage and retardation of healing. This study investigates the application of brushite and monetite calcium phosphate coatings to improve the corrosion behaviour and biocompatibility of Mg substrates.

In vitro immersion tests were carried out in Earle’s balanced salt solution (EBSS), minimum essential media (MEM) or MEM with protein (MEMP) for 7, 21 or 28 days. In vivo assessment involved the subcutaneous implantation of samples on the dorsal region of Lewis rats for 3, 6, 9 and 12 weeks. Further in vivo investigations included intraosseous implantation in both cortical and cancellous bone of Romney Cross sheep. Corrosion was assessed via gravimetric analysis or volume loss and biocompatibility was assessed histologically using a haematoxylin and eosin (H&E) stain.

In vitro immersion tests showed a maximum weight loss of 10% for naked magnesium, 6% for brushite, and 4% for monetite at 28 days. Monetite coatings improved corrosion resistance in EBSS ($P < 0.05$, n = 3) and MEMP ($P < 0.01$, n=3) compared to naked controls. A protective trend was also observed in MEM. Similarly, brushite provided significant corrosion protection ($P < 0.05$, n=3) in MEMP with a protective trend seen in other solutions. Subcutaneous investigations demonstrated monetite provided significant corrosion protection over 3 months ($P < 0.05$, n=6) with a trend towards improved resistance with brushite (all statistics performed were ANOVA with Bonferroni post-hocs). Preliminary analysis of subcutaneous and intraosseous histology illustrated that both coatings reduce corrosion and improve biocompatibility.

Our findings indicate that calcium phosphate coatings may be an effective way of improving Mg properties for future clinical application of biomaterials of this nature.
Induction of hypertension blunts baroreflex inhibition of vasopressin neuron activity in Cyp1a1-Ren2 (inducible hypertensive) rats. S Han1,2, D Schwenke1, C Brown1,2.

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When blood pressure acutely increases, vasopressin secretion decreases as part of the corrective baroreflex response. However, in some hypertensive individuals, vasopressin level is paradoxically increased, despite chronically-elevated blood pressure. Here, we investigated the mechanisms that underpin the paradoxically-increased vasopressin level using transgenic Cyp1a1-Ren2 rats, which develop moderate angiotensin II (ANGII)-dependent hypertension when fed 0.225% indole-3-carbinol (I3C) over seven days.

Extracellular single-unit recordings of vasopressin neuron firing rate were made from urethane-anaesthetised Cyp1a1-Ren2 rats fed ordinary diet (CYP) or 0.225% I3C for seven days (HD7). The vasopressin neuron firing rate was higher in HD7 rats (8.8 ± 0.8 spikes s\(^{-1}\); mean ± SEM, n = 22) than in CYP rats (6.1 ± 0.5 spikes s\(^{-1}\); n = 30; \(P = 0.004\), unpaired t-test). Intravenous injection of the \(\alpha_1\)-adrenoreceptor agonist, phenylephrine (PE, 2.5 µg kg\(^{-1}\)), inhibited vasopressin neurons in CYP rats (by 61.9 ± 8.2%, n = 15) but not in HD7 rats (15.4 ± 15.5%, n = 13; \(P = 0.01\)), despite a similar increase in blood pressure. Intra-subfornical organ (SFO) infusion of the ANGII-receptor antagonist, losartan (5 µg hr\(^{-1}\)), during the induction of hypertension did not affect the development of hypertension or the increased vasopressin neuron firing rate (8.8 ± 1.4 and 9.7 ± 1.3 spikes s\(^{-1}\), n = 17 and 36 in vehicle- and losartan-treated HD7 rats, respectively; \(P = 0.66\), unpaired t-test). Similarly, intra-SFO losartan infusion did not affect phenylephrine-induced vasopressin neuron inhibition (36.7 ± 9.1 and 28.7 ± 9.2%, n = 15 and 27 in vehicle- and losartan-treated HD7 rats, respectively; \(P = 0.57\), unpaired t-test).

Hence, induction of ANGII-dependent hypertension likely increases vasopressin neuron activity via reduced baroreflex inhibition, rather than by activation of ANGII-sensitive afferent inputs. Therefore, reduced baroreflex gain might exacerbate hypertension, in part, by increasing vasopressin-induced vasoconstriction and water retention.

Association of non-specific low back pain with physical activity, smoking and food choices in New Zealand adolescent females. N Mehta1, G M Johnson1, P Skidmore2, M Skinner1, S Milosavljevic1. 1School of Physiotherapy, 2Department of Human Nutrition, University of Otago, Dunedin.

The emerging literature indicates the trio of low PA, smoking and poor diet is associated with the most common musculoskeletal disorder, low back pain (LBP). Our aim is to investigate whether a combination of more than one can significantly increase the risk of developing non-specific low back pain (LBP) in adolescent females, which was investigated in this study.

An online cross-sectional survey was completed by 307 girls (mean age 14.8 ± 1.2 years) from six Otago schools. Data were collected on prevalence of LBP (current, within past month, 6 mth, 1 y, 3 y); smoking history (current, lifetime), PA and food choice. Participants reporting LBP on three or more distinct occasions were further classified as having recurrent LBP. PA was classified by participants meeting national recommendations for moderate to
vigorous PA (MVPA) or not. Usual frequencies of food consumption using indices for fruit and vegetables (FV), fibre foods, calcium foods, treat foods and an overall dietary variety index were calculated.

The results showed that the recurrent, past month and point prevalence of LBP were 37.6%, 25.6% and 15.2% respectively. The prevalence of lifetime smoking was 19.8%; 4.5% were current smokers. Less than one-third of the participants met the MVPA recommendations; 46% met daily FV consumption guidelines and 24.4% consumed treat foods on at least five days/week. Multiple logistic regression showed a significant association for PA, odds ratio (OR) = 1.80 [confidence interval (CI), 1.03 - 3.16, \( P = 0.03 \)] and lifetime smoking OR = 1.94 [CI, 1.03 - 3.64, \( P = 0.03 \)] with LBP in the past month, whereas their association with recurrent LBP was not significant. No significant associations were found with any food variables.

In conclusion, adolescent females who meet the MVPA criteria and are lifetime smokers have a higher risk of developing LBP irrespective of their dietary habits.