



Wellington Medical Research Foundation

Research Review 2008

**WELLINGTON MEDICAL
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Editorial

New Zealand has a proud record in biomedical research with the achievement of many important discoveries that have had a major impact upon the wellbeing of the global community. Doubtless, influenced by these successes, as well as by the high international reputation of New Zealand biomedical investigators, many of our outstanding graduates have opted for a research-focused career. Over succeeding years many research groups have been formed that have built upon earlier successes and fostered an educational environment for emerging generations of researchers.

Support for biomedical research has always been available from a number of sources, with the bulk of funding now coming from government agencies. In addition to this, infrastructural support has, in the past, been provided by host tertiary institutions in fulfilment of the requirements that, for universities, original research should underpin and inform both undergraduate and postgraduate teaching programmes.

Over recent years the funding environment for biomedical research has changed dramatically. The steady decline in funding available to universities has meant that there is now an expectation for researchers to fund their own overheads as well as their research activities. This is not always possible; however, and while government agencies do factor in overhead costs when awarding project and programme grants, voluntary funding organisations rarely provide overheads as a component of a research grant.

In recent years the real value of funds available for research through the Health Research Council, the major funder of biomedical research in New Zealand, has been eroded by inflation and the spiralling costs of supplies and equipment. This decline in funding is reflected in the gradual decrease in the proportion of applications approved for funding by the Health Research Council which reached a nadir of 17% in 2007. For the 2008 round the Council obtained an additional \$4.5 million in funding, which represents an increase of 5.9% over the previous year. In reality the increase was 4.4% as only \$3.375 million of this additional funding was made available for the contestable round. This pales somewhat when compared to the \$90 million that the government was reported to have invested in the securing of nine medals at the Beijing Olympics!

One of the goals of the Health Research Council is the investment in research that has a high impact internationally, and scientific merit is a requirement for any grant to achieve funding. However, since 2002, the Council has given priority to those applications that satisfy one or more of nine defined research portfolio strategy priorities and/or focus on any of the five priority population groups. Although it can be argued that the awarding of funds in this manner addresses health inequalities in New Zealand, it can also be argued that because of this policy the criteria for funding is shifting from a scientific to a social imperative. Doubtless this will also mean that less funds will be made available to those groups whose core activity is basic rather than social science.

The parlous state of health research funding in New Zealand was recently reinforced in a report published in the *New Zealand Medical Journal*. In this it was noted that local funding is up to twelve times less than that available in some OECD countries. More specifically it was noted that per capita funding available in New Zealand was one third that in Australia and less than one tenth of that available in the United States.

This steady decline in funding for biomedical research is already having a serious effect on staffing levels. Several established and respected research groups have been forced to cut staff and in some instances disband completely.

The poor performance of government funding agencies means that many researchers must rely on the voluntary funding sector for ongoing support and this is clearly reflected in the increasing number of grant applications received by the Wellington Medical Research Foundation. While not all applications are approved for funding, the Foundation continues to make an important contribution by supporting established research groups and thus ensuring the ongoing development of biomedical research in the greater Wellington region.

Professor Brett Delahunt
Editor

Research Advisory Committee Membership

Professor Brett Delahunt (Chair)
Professor Carl D Burgess
Associate Professor Duncan C Galletly
Dr T William Jordan
Dr Jeremy D Krebs
Professor Graham Le Gros
Professor John H Miller
Dr David Slaney

Reports of research work funded by grants prior to the current year

Capital and Coast District Health Board

Does the time of day affect the outcome of the Dix-Hallpike manoeuvre, when testing for Benign Paroxysmal Positional Vertigo (BPPV)?

A Burston
Department of Neurology

The aim of this study is to establish if there is a variation in the results of the Dix-Hallpike manoeuvre if it is performed at different times of the day.

The study is still in progress. Data collection was commenced on 10 July 2007. The intended number of participants for the study is 50. To date 39 participants have been recruited, and 39 for whom the research has been completed. No participants have withdrawn. Recruitment has been steady, with a high proportion of people referred with BPPV agreeing to take part in the study. If recruitment remains steady, the original estimated time for finishing data collection of September 2008 is still realistic.

There have been no changes to the study as outlined in the original proposal. The author and 2 supervisors have also remained unchanged.

The equipment has been working well and recording the data as anticipated. It is easily portable which enables participants to be assessed either at the Kapiti Health Centre, or in their homes if they do not have access to transport.

There have been no adverse effects other than the usual side effects in some participants, of nausea, increased sense of being off balance, and increased dizziness for a period of time following the testing. The participants are warned of the possible side effects before the tests are performed, and care is taken to support the participants until they are steady. This is standard practice and is a normal response to the testing and not due to the research.

The Use of Metformin in patients commencing atypical antipsychotic therapy

J M Wilson
Endocrine Department

Recruitment has been very slow with less than expected numbers of patients being referred from the psychiatric services. To date there has only been four participants entered into the study. One has completed the six months, two have pulled out part way through study and currently one is ongoing.

We have recently had ethics approval extended for a further year and hope to recruit the desired amount of greater than twenty participants by this time period.

Institute of Environmental Science and Research (ESR)

Mucosal Immunity: Implications for Meningococcal Carriage and Spread

JK MacKichan
Communicable Disease Group

Neisseria meningitidis (meningococcus) is one of the leading causes of infectious disease worldwide, resulting in meningitis or severe sepsis (meningococcaemia), which are often fatal. Even with prompt antibiotic therapy, the fatality rate is 10-20%, with a further 12-19% suffering long term effects, including neuronal defects or loss of limbs or hearing. In spite of this, the natural habitat of meningococcus is the human throat, where it rarely causes disease or symptoms. About 8-25% of the general population carry meningococcus asymptotically. Occasionally, highly virulent (*e.g.*, disease-associated) strains can emerge and cause widespread outbreaks or epidemics, as occurred in New Zealand beginning in 1991, in contrast with strains primarily associated with carriage or sporadic disease (*e.g.*, carriage-associated). Genetic differences between strains likely underpin observed differences in behaviour, but have yet to be identified and remain a significant unknown.

Invasive disease occurs when meningococcus in the throat gains access to deeper host tissues, including the bloodstream. The means by which this process occurs are unclear. The lack of animal models for carriage and invasive disease has hampered study in this area, and *in vitro* assays have to date provided limited insights. Meningococcus lacks many of the canonical virulence factors (*e.g.*, secretion systems or toxins), and nearly all of the “virulence factors” identified to date (*e.g.*, type IV pili) enable meningococcus to colonise its host, but typically are equally present in disease- and carriage-associated strains. Using the extensive collection of meningococcal strains at ESR, the genetic and phenotypic differences between carriage- and disease-associated strains were examined.

Using an *in vitro* model of meningococcal infection of host epithelial cells, striking differences in behaviour between carriage- and disease-associated strains were noted. Following 24 hours of *in vitro* infection, a subset of disease-associated strains caused extensive destruction to the epithelial cell monolayer, a phenomenon not observed for carriage-associated strains. This observation implies there is a previously uncharacterised bacterial factor in disease-associated strains with potential cytotoxic activity, a finding that has implications for further understanding of the pathogenesis of meningococci. An analysis of the genetic differences between selected strains was performed by subtractive hybridisation, which allows identification of genetic elements present in one strain (a “tester” strain) but absent in another (the

“driver” strain). Genetic elements present only in the strains with cytotoxic activity have been identified, and further work to analyse the function of these genes, by deletion analysis, is ongoing. In parallel, the generation of a transposon mutant library in a disease-associated meningococcal strain will allow for a functional screen for mutants that have lost the cytotoxic activity.

Malaghan Institute of Medical Research

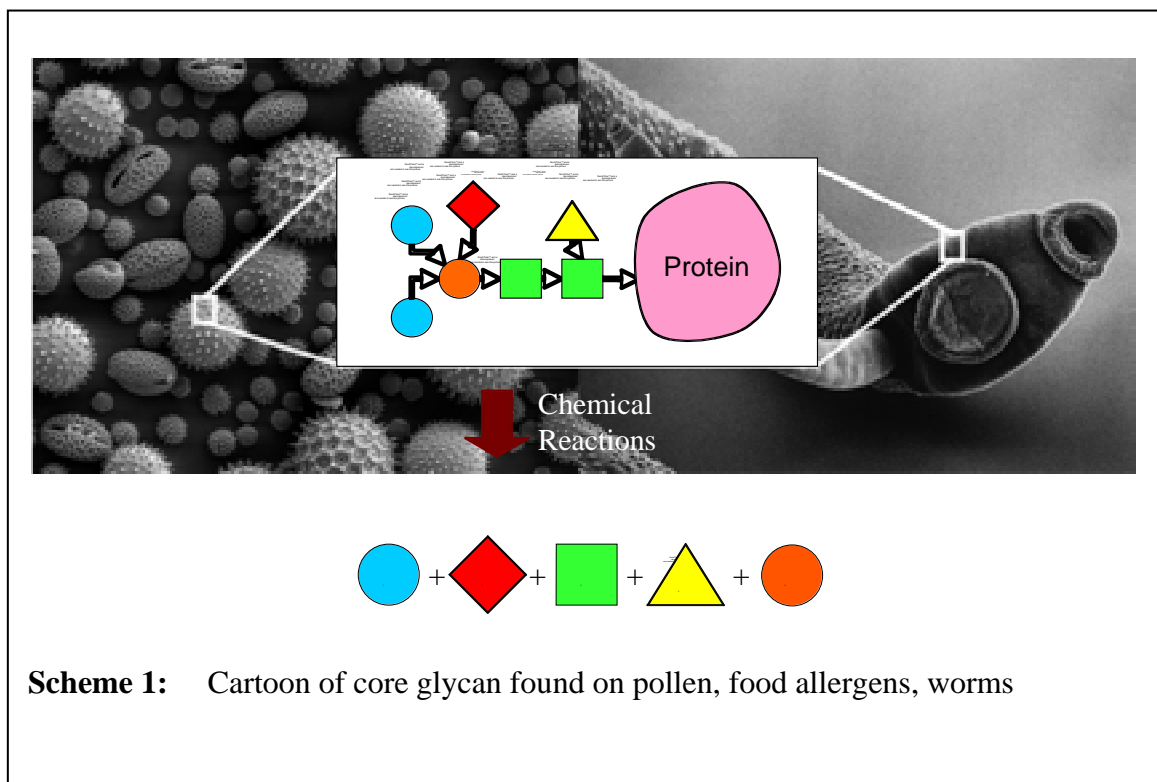
A Sweet solution to Th2 bias in Asthma

B L Stocker

Asthma is an allergic disease caused by a hypersensitivity of the immune system to external allergens resulting in a strong T helper cell (Th) 2 response. Much is known on the macro-molecular level about the causes of asthma, though few studies have looked at the molecular structure of these allergens and the role that this “micro” structure plays in influencing a Th2 bias. In the past, significant emphasis has been placed on the role of the protein constituent of the allergen and the appended sugar (carbohydrate) motifs have been largely overlooked. The objective of this research is to investigate the influence these complex carbohydrate structures have on immune (Th1 or Th2) bias. Specifically, by testing a range of synthetic carbohydrate probes, we aim to identify key carbohydrate structures responsible for a Th2 bias, which in turn will lead to a better understanding of allergy and asthma.

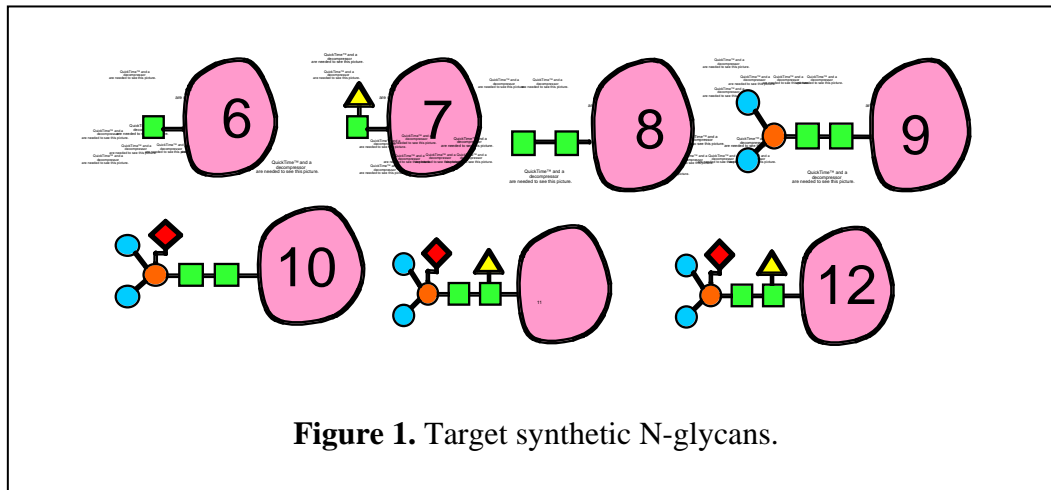
This research programme is anticipated to take 3 years. Funding from WMRF has been used to initiate the synthesis component of the programme and, following the initial WMRF grant, further funding (Lotteries, HRC) has been obtained to see the continuation of this programme. Over the past year, through WMRF support, excellent progress has been made toward this project. The key building blocks required for the assembly of the glycans have been prepared, provisional coupling studies performed (leading to a number of simple glycans that will soon be tested *in vivo* for their Th2 skewing abilities), and studies looking into the optimisation of key synthetic transformations have been undertaken. Details of these outcomes are provided below. Due to the commercial sensitivity of this work, cartoons have been used to depict the required glycans.

a) Synthesis of key building blocks: As outlined in Figure 1 (overleaf), synthesis of the most complex glycan requires five suitably protected carbohydrate building blocks, 1 – 5. Each building block takes between 2 – 8 steps to prepare. It takes approximately one month to establish a reproducible protocol for each building block. Synthesising gram quantities of the final building blocks takes an additional two weeks to two months.



Synthesis of building block 1 has been completed. This is a seven-step synthesis (with an overall yield of 44%), and to date, approximately 3 grams of the building block (sufficient for the duration of this project) has been prepared. Building block 2 is the most time consuming building block to prepare, taking eight steps. The synthetic pathway for the synthesis of this building block has been established and the building block is currently being prepared on the multi-gramme scale. The scaled-up synthesis of 2 is anticipated to be completed by July 2008. Building block 3, prepared in 2 steps with an overall yield of 33%, has been synthesised in approximately 1.5g. Similarly, building block 4 (5 step synthesis with a 56% overall yield) has been prepared in a 1.5g quantity. Finally, building block 5, can be prepared in 6 steps, with a 63% overall yield. 7 g of this building block has been prepared.

b) Toward construction of the key N-glycans: These building blocks are then combined in the appropriate manner to form the target glycans, 6 -12, (Figure 1). Monomer 6, coupled to OVA, has been synthesised and, following conformation of structure by Matrix-assisted laser desorption/ionization (MALDI) mass spectrometry, will shortly be tested *in vivo* for its Th2 skewing abilities. The precursor to glycan 7 (the unconjugated, fully protected disaccharide) has also been prepared, though improvements to the efficiency and $\alpha:\beta$ selectivity of the glycosylation reaction between building blocks 3 and 4 need to be made. Methodology for the deprotection of the protected disaccharide is currently being investigated. Disaccharide 8 will be synthesised within the next month, while the more ambitious structures, 9, 10, 11 and 12 will be prepared early 2009.



c) Optimisation of key reactions: Optimising linking the carbohydrate portion to the protein is key in synthesising the target glycans. Literature conditions, through giving the required linkage, take several weeks to perform and are not optimal for the fictionalisation of larger structures. A number of novel protocols have been investigated, leading to reaction times being reduced to several days and improvements in yields.

Immune Inflammation in Neutrophilic disease – A study of Gouty Arthritis

R Grainger and JL Harper

Gout is one of the most frequently reported diseases in history and causes significant morbidity in affected individuals. Despite this, it has been largely ignored in biomedical research and the pharmaceutical industry, severely limiting the number of treatments for gout sufferers. The incidence of gout is rising overseas and in New Zealand, and is disproportionately high in New Zealand within our Maori and Pacific Island population. Combined with an increase in the number of patients with refractory disease, this highlights an urgent need to improve disease management. This can be achieved through a better understanding of the inflammatory mechanisms behind a gout attack. Gouty arthritis is triggered by the formation of monosodium urate crystals (MSU) in the joints and/or soft tissue leading to inflammation and in cases of severe chronic disease joint erosion. Although formation of MSU is associated with elevated levels of uric acid in the blood having high uric acid (hyperuracaemia) does not automatically result in gouty disease. In fact 80% of hyperuraceamic individuals remain asymptomatic.

One of the key characteristics of gouty inflammation is the infiltration and activation of neutrophils leading to the production of the neutrophil chemokine IL-8 and the reactive oxygen species superoxide. The aim of this study was to isolate the neutrophils from the blood of patients with chronic or acute gout, asymptomatic hyperuraceamics and healthy volunteers and compare the inflammatory responses of these cells following exposure to the gout-causing

agent MSU. These data were then analysed to identify if a heightened immune response to MSU was associated with susceptibility to developing gout.

Neutrophils isolated from blood and exposed to MSU *in vitro* produced comparable high levels of the neutrophil chemokine IL-8 across all study groups.

Neutrophils from patients with hyperuricaemia (asymptomatic, acute and chronic gout) produced higher levels of the reactive oxygen species superoxide at lower concentrations of MSU compared to healthy controls indicating that hyperuricaemia may increase sensitivity to MSU. Surprisingly there was difference in superoxide production between the different patient groups. These data provide preliminary evidence that uric acid levels in the blood alters the inflammatory phenotype of neutrophils. This may contribute to the susceptibility of individuals to developing gout however the comparable responses between gout patients and asymptomatic hyperuricaemics indicate that other factors this is not a sole marker of susceptibility.

NK-T Cell Activation And Dendritic Cell Survival In Vivo

H A Simkins and F Ronchese

NK-T cells are a population of T cells expressing an invariant T cell receptor and markers of NK cells such as CD56 in humans and NK1.1 in mice. Upon intravenous injection of the appropriate antigen ligands *in vivo*, NK-T cells become activated and rapidly produce cytokines that lead to the activation of other immune cell populations, and support the establishment of immune responses.

Intravenous injection of the NK-T cell ligand α -galactosyl ceramide (α -GalCer) is known to induce activation of dendritic cells, a population of powerful antigen presenting cells that are critical to the initiation of immune responses. We have observed that activation of dendritic cells after α -GalCer injection is followed by a substantial reduction in a subpopulation of dendritic cells that carry the surface marker CD8. Other dendritic cell subpopulations do not appear to be as profoundly affected.

To determine whether the reduction in dendritic cell number after α -GalCer injection requires direct contact between NK-T cells and dendritic cells, we carried out experiments where α -GalCer was loaded on dendritic cells in culture, and these dendritic cells were then injected intravenously into mice to induce NK-T cell activation. Surprisingly, we found that injection of dendritic cells already loaded with α -GalCer caused a decline in the number of CD8+ dendritic cells, regardless of whether the injected DC were able to directly activate NK-T cells or not. These data suggest that presentation of α -GalCer by CD8+ dendritic cells, which are very efficient at taking up and presenting materials injected intravenously, may be a critical event in the disappearance of the CD8+ dendritic cells themselves.

We also examined the role of cytokines in the reduction of CD8+ dendritic cell numbers. Injection of α -GalCer is known to induce NK-T cells to rapidly secrete TNF- α , which induces initial activation and then death of dendritic cells *in vitro*. Injection of an antibody that neutralizes TNF- α was effective at reducing the serum levels of this cytokine in treated mice. The numbers of CD8+ dendritic cells were also less drastically reduced in mice that received anti-TNF compared to the mice that did not, suggesting that TNF- α is involved in the death of dendritic cells during α -GalCer treatment. The role of IFN- γ was also examined using IFN- γ deficient mice, unfortunately these experiments were inconclusive and no effect of IFN- γ could be convincingly demonstrated.

In conclusion, we have started to define some of the factors that affect the activation, survival and death of CD8+ dendritic cells during treatment with α -GalCer. The use of α -GalCer as a powerful adjuvant for the induction of T cell and B cell immune responses, and anti-tumour immune responses, has received much interest. We hope that by improving the survival of dendritic cells *in vivo* during α -GalCer treatment, the ability of α -GalCer to induce immune responses will also be improved.

Massey University

Akt as a therapeutic target for muscle wasting induced by acidosis

JA Edge and MJ Short
Institute of Food, Nutrition and Human Health

The loss of skeletal muscle is a serious clinical problem due to its relationship with morbidity and mortality. Many conditions such as chronic kidney disease, cancer, sepsis and insulin deficiency result in increased breakdown and loss of skeletal muscle. Metabolic acidosis accompanies a number of disease conditions such as chronic kidney disease and some insulin disorders. Metabolic acidosis has been shown to have a negative effect on skeletal muscle development and accelerate muscle degradation in animal models. In contrast, regular physical activity promotes the maintenance and growth of skeletal muscle and may provide protection against many disease related muscle wasting disorders. To date few studies have examined the effects of metabolic acidosis on human muscle and whether physical activity has a protective effect against the negative consequences of metabolic acidosis. A key signalling protein involved in the regulation of skeletal muscle development is Akt. Therefore, identifying changes to activation of this protein following metabolic acidosis and physical activity may provide insight into how metabolic acidosis affects skeletal muscle development and determine how long term involvement in exercise may be of benefit to skeletal muscle.

The aim of this project was to determine the effects of (1) short-term metabolic acidosis on the molecular response (ie phosphorylation of Akt) of skeletal muscle in healthy humans and (2) the effects of the physically trained

status of skeletal muscle on the molecular response during metabolic acidosis.

Sixteen healthy humans aged 38 – 60 y volunteered to participate in this study. Eight of the participants were healthy and active (Mean \pm SD, age 45 ± 9 y, BMI 27.6 ± 3.8 kg/m²), while the other eight were well-trained endurance athletes (age 44 ± 8 y, BMI 24.2 ± 1.9 kg/m²) who had been training regularly (4 – 6 d·wk⁻¹) for >10 y. Prior to the experimental procedures of the study participants undertook girth and skin fold measures to determine lean thigh volume, a strength test to determine leg strength and a graded exercise test to determine maximal oxygen uptake (aerobic fitness).

Participants reported to the laboratory 4 h after consuming a standardised light meal. After a 30 min rest period, participants had a venous blood sample taken from an antecubital vein and a muscle biopsy was taken. They were each given food packages that contained all of their food to be eaten over the following 72 h. Therefore, each participant was on the same diet, with portion sizes being the only difference between them. Participants were also given a set of tablets containing ammonium chloride to induce metabolic acidosis to be taken over the following 72 h. The participants were required to refrain from participating in formal exercise for 24 h prior to the first biopsy and until the study was completed 72 h later. Approximately 72 h (± 1 h) after their first blood sample and muscle biopsy the participants returned to the laboratory for a second blood sample and muscle biopsy 4 h after their last provided meal.

The well trained runners had a significantly higher maximal oxygen uptake than the active but not well trained participants (60.5 ± 4.0 vs 47.1 ± 4.3 mL·kg⁻¹·min⁻¹ respectively; $P < 0.05$). There were no differences in lean thigh volume or leg strength between the two groups ($P > 0.05$). The venous blood samples are currently being analysed for pH, bicarbonate, glucose and insulin levels. The muscle samples are currently being analysed for activation (phosphorylation and mRNA) of proteins involved in muscle growth (Akt, Insulin like growth factor-1, Mammalian target of rapamycin and p70 S6k1) and muscle degradation (atrogin-1 (also known as MAFbx) and muscle ring finger).

The results from this study will allow us to determine the effects of short-term metabolic acidosis on the activation of key muscle proteins involved in the maintenance and breakdown of skeletal muscle. We will also be able to determine if a chronic exercise history (i.e. exercise training background) provides a protective effect against any possible negative consequences that occur due to metabolic acidosis. The identification of molecular changes within muscle during conditions such as metabolic acidosis will allow further advancement and understanding of appropriate therapeutic targets to combat muscle wasting.

University of Otago Wellington, School of Medicine and Health Sciences

Assessing insulin sensitivity and glucose excursions in patients with type 2 diabetes in response to altered macronutrient composition in the diet

D Bell and J Krebs
Department of Medicine

We are undertaking this research as a sub study of a multicentre HRC funded trial, the DEWL study. Wellington is the major centre coordinating this trial, there were 188 participants recruited in Wellington, and 419 recruited in total.

Specific objectives

- To assess whether a high-protein: low-carbohydrate diet is more effective than a low-fat: high-carbohydrate diet in improving insulin sensitivity in patients with type 2 diabetes over 1 year.
- To compare the glucose excursions in patients with type 2 diabetes on the different diets via the 72hr continuous glucose monitor.

We have currently performed continuous glucose monitoring and oral glucose tolerance testing on;

- Baseline 30 people
- Six months 14 people,
 - Three people have had failed sensors or missed appointments but are still in the study
 - Five participants have withdrawn
 - Eight participants are booked for their CGMS in the next 6 weeks.
- 12 month CGMS bookings start at the end of July 08.

We had aimed to have 50 participants, from our power calculations looking for a 10% difference in insulin sensitivity there would need to be 25 people in each arm of the study to meet the 80% power and $P < 0.05$. We were unable to recruit 50 people for the study, and we have so far had a drop out rate of (5/30) 17%.

Noting the above numbers of people it may be that our study will be under powered to identify a possible difference between the two groups of individuals in each arm of the dietary intervention, but it will still provide information on insulin sensitivity and glycaemic excursions within these groups. It is as yet unknown the proportion of the remaining 25 participants as to the dietary intervention they are on, as we can not unblind the DEWL study at this point.

There is currently a study being performed by the ADA and IDF, to look at an equation for using the measured HbA1c to provide an estimated Average

Blood Glucose, eABG. This study is using both CGMS and finger prick glucose measurements in 600 people. Our study will be well placed to comment on their version of the average blood glucose estimation in a group of people undergoing a dietary intervention, and also looking at how HbA1c reflects that change over a year.

Early Vascular Disease in Children with Epilepsy Receiving Anticonvulsants

NF Keenan, LG Sadleir and EJ Wiltshire
Department of Paediatrics

The earliest stages of atherosclerosis begin in childhood and include detectable arterial endothelial and smooth muscle dysfunction and arterial wall thickening. These can be reliably measured non-invasively, providing an opportunity to determine the important risk factors for progression of disease during childhood and adolescence, at an age when intervention is more likely to have a beneficial effect on long-term vascular health. Adults with epilepsy have been shown to have a three-fold increase in cardiovascular death. Anticonvulsant medications in children are associated with hyperhomocysteinemia a known risk factor for endothelial dysfunction and subsequent atherosclerosis. This study will produce evidence on whether vascular function and total plasma homocysteine levels are altered in children with epilepsy receiving anticonvulsants compared to healthy controls.

Children with epilepsy between the ages of 8 and 17, who have received unchanged anticonvulsants for over 12 months, are actively being recruited across the Wellington and Hutt Valley area. Each child with epilepsy will be age, sex and BMI matched with a healthy control. The children and controls will each have their vascular endothelial health assessed using flow mediated dilatation (FMD), glyceryl trinitrate dilatation (GTN), and carotid artery intima media thickness (c IMT), aortic intima media thickness (aIMT). Each child will also have a fasting blood sample taken measuring serum cotinine, glucose, lipids, total plasma homocysteine, serum folate, RBC folate and Vitamin B6 and B12. We aim to recruit a sample size of 30 participants with epilepsy and 30 controls. This sample size gives a power of 80% at the 5% level of significance to detect a difference in FMD of $2.9 \pm 4\%$ between children with epilepsy and controls (i.e. 9% increase in FMD in controls verses 6.1% in children with epilepsy).

We have been recruiting actively since January 2008 and have recruited 19 children with epilepsy and 4 controls. This is at a rate of 3 participants with epilepsy per month which will allow recruitment of 30 participants to be complete in October 2008. Controls are being recruited by encouraging participants with epilepsy to bring a friend of the same age, sex and of similar size along to the testing. Testing started in April 2008 and we have currently tested 8 participants. Testing occurs 1 or 2 times a week in the mornings at 7.30am. This testing rate allows us to complete testing in December 2008. However, the sonographer working on the research is planning to train up a

second sonographer to allow this to become more frequent. No data has yet been analysed at this stage.

Research with the SidePak AM510 Personal Aerosol Monitor

R Edwards, N Wilson and G Thomson
Department of Public Health

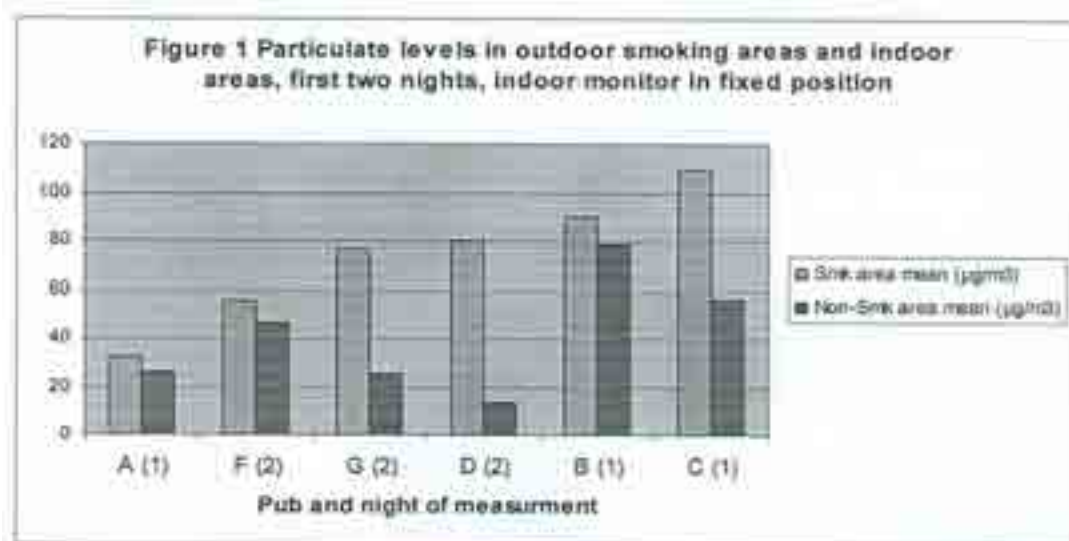
This is the second report for an equipment grant to purchase a *TSI SidePak AM510 Personal Aerosol Monitor*. The Aerosol Monitor will be used to support a programme of research investigating the level of hazardous fine particulate pollution from secondhand smoke in a variety of settings including private cars, semi-enclosed outdoor areas and homes.

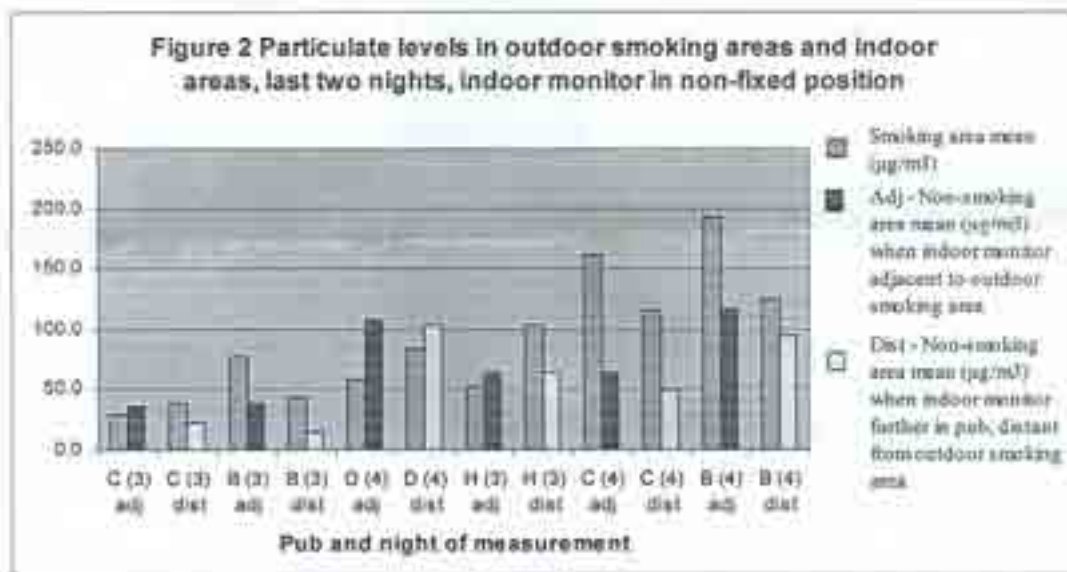
The equipment was required for use in research projects and as a component of research grant applications to support work investigating levels of secondhand tobacco smoke (SHS) in a variety of settings.

1. Investigation of levels of SHS during simultaneous measurements in semi-enclosed outdoor smoking areas and adjacent indoor areas of pubs and bars in Wellington CBD.

We have since then carried out further data collection for this project, including carrying out measurements during Summer evenings, and varying the protocol so that the indoor monitor was moved from adjacent to the outdoor semi-enclosed smoking area to further inside the pub half way through data collection.

The key results are shown below.





Our conclusions were:

- Air quality in semi-enclosed outdoor smoking areas was very variable, and in some was very poor.
- Where free communication exists between outdoor smoking areas and indoor areas, SHS drift can greatly reduce indoor air quality throughout the pub.
- Regulations to restrict the degree of communication and proximity of smoking areas to indoor areas may be justified to maintain air quality.

2. Grant application for work on SHS levels in homes with varying smoking policies

Our application for a HRC grant for this aspect of work was unsuccessful. We will work with the Housing and Health programme within the Department of Public Health to seek other possible avenues to fund this work.

3. Air quality effects due to road traffic pollution and SHS smoking in outdoor areas

We have carried out a pilot study. involving testing air quality in the Mount Victoria tunnel, in order to compare the particulate levels due to road traffic pollution and SHS pollution in enclosed outdoor areas. We need to do some further calibration work before we are in a position to finalise this study.

TLR gene polymorphisms in the NZ Asthma and Allergy Birth Cohort

Julian Crane¹ and Rod Lea²

¹Department of Medicine, ²Institute of Environment Science and Research

The aim of this project is to examine the interactions between toll-like receptor (TLR) gene polymorphisms and domestic endotoxin exposure on the early development of wheezing and eczema and on the later development of atopy and asthma in ~1000 New Zealand children.

To date we have completed genotyping of the specified TLR polymorphisms for ~ 2/3 of the cohort. Preliminary statistical analysis of genotype/phenotype data has provide some suggestive evidence of a statistical interaction which is in support of our primary hypothesis that endotoxin exposure may be associated with wheezing and protect against atopy amongst certain TLR phenotypes.

The field work for the study was only complete in Christchurch in January and we have finally collected all of the remaining DNA samples and sent them to ESR for genotyping. We are pleased to say we are on track to complete this project within the 18 months as planned with the genotyping phase of the project finishing in September 2008 and the final analysis and report completed by December 2008.

Victoria University of Wellington

Cell division as a source of new anti-tubercular drug targets

R O'Toole

School of Biological Sciences

In 2006 alone, 1.7 million people died from TB and an estimated 9.2 million new cases of the disease were reported. The World Health Organisation (WHO) estimates that in the years 2000-2020, approximately 35 million people will die from TB. In New Zealand, the incidence of TB is approximately double that of Australia and the USA and rates among Maori and Pacific Islanders are 5 and 12 times higher respectively, than that of NZ Europeans.

There is currently an urgent need for the development of new drugs to provide a cure for infections with multi-drug resistant strains. In addition, drugs that could reduce the anti-TB treatment period (currently 6 to 9 months) or could eliminate latent TB infections would aid significantly in reversing the global incidence of TB.

The current anti-TB treatment regimen targets primarily proteins involved in cell wall biosynthesis, transcription, or translation. The cell division process has received only modest attention for antibacterial drug discovery to date. Cell division is an essential process in all living organisms and is markedly

different between prokaryotes and eukaryotes. Its promise as a source of new drug targets is supported by the recent discovery of Zantrins, antagonists of the cell division protein FtsZ, which have been shown to be lethal towards several important clinical bacterial pathogens including methicillin-resistant *Staphylococcus aureus* and *Streptococcus pneumoniae*.

The primary objective of this work is the discovery of compounds which are effective against mycobacteria using cell division as a source of bacterial-specific drug targets.

In our work to date, we have identified a set of genes which have a predicted function of regulating or effecting cell division in *Mycobacterium tuberculosis*. We have identified the orthologues of these genes in the non-pathogenic species *Mycobacterium smegmatis* and have validated the essential role of a number of these proteins in mycobacterial cell division.

To detect anti-mycobacterial activity of chemical ligands, we have developed a rapid high-throughput fluorescence-based assay. We are applying this assay to the screening of chemical libraries, in particular the Library of Pharmacologically Active Compounds (Lopac, 1280 compounds) and the Spectrum Collection (2,000 compounds). In addition, the development of the assay has led to collaborations with researchers at the University of Auckland, University of Waikato and Victoria University of Wellington who have provided novel natural products for anti-mycobacterial testing. To date, from our chemical screens we have identified compounds which completely inhibit mycobacterial growth at concentrations of 15 μ M and lower. The compounds will be tested for cytotoxic activity to derive selectivity indices.

In addition to identifying compounds which inhibit mycobacterial growth, we are currently using a technique known as antisense differential sensitivity to determine compounds whose mode of action entails disruption of cell division. In particular, from the series of compounds that we have found to block mycobacterial growth, we are using antisense differential sensitivity to identify those compounds which block the function of specific cell division proteins.

The research outcomes of this project are two-fold: the project has applied recently-developed genetic techniques for (i) the identification of new drug targets in mycobacterial cells; and (ii) the linking of these individual genes with their respective chemical inhibitor.

Effects of Tobacco Smoke Components on Nicotinic Receptors, Monoamine Oxidase Enzymes and Mu-Opioid Receptors in Cultured Neuroblastoma Cells

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Cigarette smoking increases the risk of developing diseases such as stroke, hypertension, cancer, asthma, and emphysema. Nicotinic receptors in the brain mediate some of the addictive behaviours associated with smoking; however, nicotine replacement therapies have proven to be only marginally effective in aiding long-term quitting. This highlights the complex nature of tobacco dependence and suggests that factors beyond addiction to nicotine may also contribute to tobacco addiction. Although limited information is currently available on the effects of non-nicotinic components of tobacco smoke, it is well known that the activity of monoamine oxidase (MAO), a key player in the neurochemistry of many behavioral traits, is significantly inhibited in tobacco smokers. These enzymes play an integral role in the breakdown of dopamine and other neurotransmitters and are implicated in addiction and reward pathways in the brain. The aim of our study is to identify and characterize the contribution of non-nicotinic compounds in tobacco particulate matter (TPM) to relapse following successful smoking cessation.

It is known that TPM elevates expression of nicotinic receptors on the cell surface more than expected from the amount of nicotine in the TPM. Thus, some other component of TPM must be interacting with cells to cause this effect. Because nicotine's ability to upregulate its receptors is believed to be integral to its addictiveness, it seems likely that this unknown component of TPM also contributes to smoking addiction. It is therefore important to characterise this bioactivity in TPM further. We are using cultured human neuroblastoma cells (SH-SY5Y cells) to identify specific changes in nicotinic receptor and MAO gene and protein expression as a result of exposure to tobacco smoke extracts from normal and denicotinized cigarettes.

With regard to MAO activity, we have found that both standard and denicotinized tobacco extracts inhibit MAO activity in cells; whereas, nicotine on its own does not. This inhibition by TPM and denicotinized TPM was also observed in purified enzyme preparations. Inhibition of MAO-A activity was significant even when TPM had been diluted to physiologically relevant concentrations as judged by the concentration of nicotine. Quantitative RT-PCR showed no significant decrease in MAO gene expression in SH-SY5Y cells after exposure to TPM; however, some upregulation of MAO-A was seen, and this may indicate compensation for the enzyme inhibition by TPM. The inhibition of MAO-A enzyme activity without a change in mRNA suggests that the MAO inhibition observed was a result of inhibitory compounds acting directly on the enzymes and not through reduced MAO gene expression. We concluded that TPM contained non-nicotinic inhibitors of MAO enzyme activity, and that these inhibitors may play a role in the addictive properties of tobacco smoke.

Mu opioid receptor activity has also been implicated in the addiction mechanisms of many drugs of abuse and has been linked to tobacco in the literature. Thus, we are also examining the effects of TPM on opioid receptor mRNA and protein expression in our cultured neuroblastoma cells. Results so far indicate that MOR is upregulated following exposure to TPM from standard cigarettes and TPM from denicotinised cigarettes, but not to nicotine alone.

In addition to the cultured cell studies, we are investigating MAO activity in blood samples collected from smoking and non-smoking volunteers. The question we are asking is whether there are clear differences between smokers and non-smokers, and this information will be used in later studies to determine if MAO expression is correlated to the ability of smokers to quit their habit. Thus, our aim is to provide a biochemical rationale to assess what factors most closely influence the success of nicotine replacement therapies.

Expression of opioid receptors in the proliferative zones of the developing nervous system

DJ Day, JH Miller, TJ Sargeant and DF Foo
School of Biological Science

Mu opioid receptor ligands such as morphine and met-enkephalin are known to modulate normal brain development by perturbing gliogenesis and inhibiting neuronal proliferation. Surprisingly, the distribution of the mu opioid receptor (MOR) in the embryonic brain, especially in proliferative regions, is poorly defined and subject to conflicting reports. Using an immunohistochemical approach, we found that MOR protein was strongly expressed in the neuroepithelia of the lateral ventricles, third ventricle and aqueduct within the late embryonic (E18.5) mouse brain. In contrast to the ventricular neuroepithelia, the proliferative external granule layer of the embryonic cerebellum did not express MOR protein, although the Purkinje cell layer did. Dividing radial glia (PCNA-positive; GLAST-positive) within the ventricular neuroepithelium expressed MOR, while migrating neuroblasts (doublecortin-positive) did not. BrdU labelling of dividing cells showed an anterior to posterior gradient of proliferation ($P < 0.05$), while an opposing posterior to anterior gradient of mu opioid receptor expression ($P < 0.05$) was found. The localisation of MOR immunoreactivity within the embryonic ventricular neuroepithelia is consistent with a role for opioids in modulating radial glial function in neurogenesis.

Long-Term Cellular Effects of Ecstasy - Adaptation or Degeneration?

JH Miller, S Schenk, DJ Day, BM Kivell, P Bosch, J Colussi-Mas, E Daniela, K Danielson, D Gittings, L Hely, B Lake and B Simonson
School of Biological Sciences and School of Psychology

MDMA (ecstasy) is a recreational drug that produces feelings of euphoria as a result of serotonin and dopamine overflow from nerve terminals. The long-term detrimental effects of MDMA are not known. We are using an animal model of drug self-administration to investigate the causes drug-seeking behaviour and relapse after abstinence and the neurochemical changes that occur in the brain that underpin those behaviours. Understanding the neurochemical changes will help us determine whether brain function can recover following long-term use of MDMA.

Using an MDMA self-administering rat model, Professor Sue Schenk has led an investigation of the effect of sensitization to the drug on self-administration behaviours and the role of dopamine and serotonin in those responses. From these studies, it is clear that MDMA self-administration, like self-administration of other drugs of abuse, is dependent on the activation of dopaminergic substrates. One of the factors that leads to relapse includes exposure to other psychostimulant drugs, and this is relevant to human drug-taking behaviour since most addicts are multiple drug users. Our initial work is encouraging since it has suggested that long-term changes in serotonin and dopamine neurotransmission in the brain exhibit recovery with extended abstinence from MDMA.

In association with Professor Schenk's group, Drs Miller, Kivell, and Day have been correlating the behavioural effects of self-administration with specific neurochemical changes in brain areas linked to the MDMA-induced hyperactivity and self-administration responses. Specific pathways under investigation include Fos-associated transcription factor activation, to highlight specific brain regions activated by MDMA exposure, and serotonin and dopamine transporter function, to determine the mechanism of the changes in levels of serotonin and dopamine neurotransmitters following exposure. Functional serotonin transporter expression decreases after longer-term MDMA exposure, and our studies suggest that this neurochemical deficit results from inactivation by internalization of the transporter in the cells (via changes in protein trafficking), rather than decreases in gene activity or protein synthesis of the transport protein. We are currently studying levels of p38 MAPK expression and activated phospho-p38 MAPK in cultured cells exposed to MDMA to determine the mechanisms behind these drug-induced changes in SERT trafficking.

A more detailed understanding of the behavioural effects of MDMA self-administration and relapse behaviours in rats and their associated neurochemical changes may lead to a rationale for promoting recovery from chronic MDMA use in humans.

Mycobacterium Tuberculosis: The fat behind the cough

MSM Timmer

School of Chemical and Physical Sciences

Tuberculosis (TB) is the greatest infectious disease scourge in the world today killing more people than any other infectious disease. Many of the difficulties that exist in finding adequate treatment for TB arise from an insufficient understanding of the fundamental processes that underlie the immune response to mycobacterial infection and immune evasion. To this end, we are looking at the role that components of the mycobacterial cell wall have on the pathogenesis of TB. The mycobacterial cell wall is composed of a number of glycolipids, differing across bacterial strains. Due to the vast array of glycolipids present, it is difficult to isolate pure materials in order to investigate the role that the glycolipids play in the immune response. To circumvent these problems, we will synthesise a variety of glycolipids and test these for their immunodulatory properties, thus aiding in the understanding of mycobacterial infection and in the treatment of TB.

To study the role of lipids in mycobacterial infection, homogeneous compounds with intra- and inter-species varying functional groups and chain lengths need to be synthesised. In year one of this three-year research programme, the focus has been on the development of a generally applicable, efficient synthetic strategy for the construction of a fairly large collection of compounds. Significant results have been obtained towards the construction of such a lipid “library”.

In general, synthesis of the relevant glycolipids can be divided into two sections:

synthesis of the carbohydrate portion
synthesis of the lipid portion

a) Synthesis of the carbohydrate portion: Studies into the synthesis of the carbohydrate portion of our glycolipids has been performed. This can be achieved followed standard literature protocol. At present, two steps are required to see the completion of the carbohydrate building block.

b) Synthesis of the lipid portion: Synthetic methodology has been developed for the synthesis of various structural motifs present in the lipid chains. These structural motifs are present in specific stereochemistries and require the incorporation of correct chiral centres at certain insertion points along the chains. To synthesise the more challenging of these functionalities, an enzymatic desymmetrisation strategy was envisioned. This strategy involves an initial four-step synthesis, followed by the desymmetrisation protocol. This four-step strategy has been completed, in good yields and in high purity, and the large-scale production of the key, prochiral, starting material has been developed. This will provide sufficient material to allow for the synthesis of all lipid analogues. The enzymatic desymmetrisation is currently under investigation and several enzymes, e.g. porcine pancreatic lipase (PPL), will be tested. Following this enzymatic resolution, three steps are required to see the completion of this portion of the lipid.

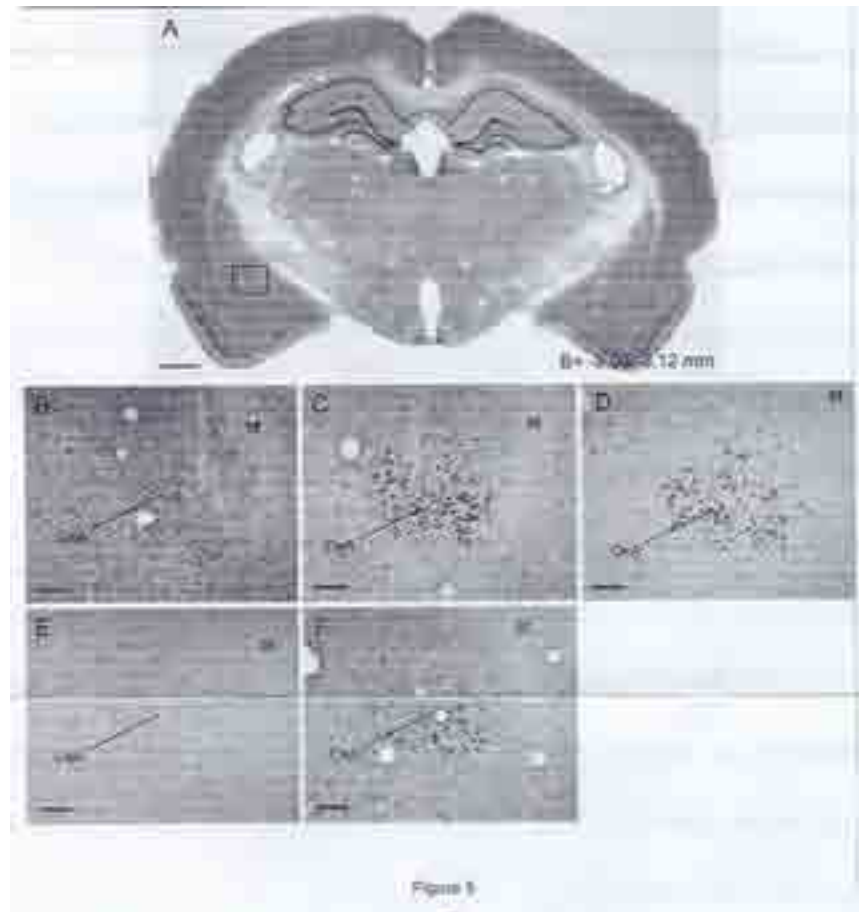
Good progress has also been made towards the synthesis of another key functionality of the chiral backbone of lipid. In this work, a chiral starting material has shown promise for the incorporation of the correct stereochemistry at the centres of the required lipid. To date, five steps of the required eight-step synthesis have been completed, in good yield, and on the gram scale. Alkylation of this 'five-step' intermediate is underway.

Neuroadaptations associated with +/- 3,4 methylenedioxymethamphetamine (MDMA, "ecstasy") exposures

S Schenk
School of Psychology

Our laboratory has established a protocol whereby MDMA is intravenously self-administered by rat subjects. Because self-administration is a reliable indicator of abuse potential we are in a unique position to investigate the neurochemical consequences of exposure. In one study we measured the density of serotonin transporters in brain tissue of rats that had received exposure to MDMA via self-administration. For these rats, there was a considerable decrease in the density of these transporters that persisted for 2 weeks following exposure. We suggested that these deficits might underlie some of the persistent behavioural and cognitive deficits that are characteristic of MDMA abusers.

In a follow up study, we measured the behavioural response to MDMA following single or repeated exposures to MDMA in laboratory rats. We showed that the locomotor activating effect of MDMA increased with repeated intermittent exposure. When the brain tissue from these rats was subsequently analysed, we observed that the increase in immediate early gene expression in a small subset of the 64 neural sites measured was correlated with the behavioural data. An example of immediate early gene expression in the central nucleus of the amygdala is shown in the figure. Results of this intensive mapping study provided an indication of brain sites that might undergo behaviourally relevant neuroadaptations and have paved the way for other experimental protocols to follow-up on these novel findings.



Opioids and brain development

DJ Day, JH Miller, T Sargeant and R Steel
School of Biological Sciences

The antiproliferative effects of opiate exposure on neurogenesis *in vitro* have been well documented, but the effects of opiates on brain development *in vivo* are less well understood. Morphine effects are mediated predominantly through its activation of the mu opioid receptor (MOR). We have recently shown that mu opioid receptors are expressed on neuronal precursors (radial glia) within the developing cerebral cortex which lead us to speculate that endogenous or exogenous ligands that act through MOR may alter brain development. In the present study we show that *in vivo* morphine treatment of the E15.5 mouse increases the length of G₂/M phase of the radial glial cell cycle in the cortex, by slowing migration of radial glial nuclei from the basal ventricular zone to the apical surface, a process termed interkinetic nuclear migration (INM). INM is an essential part of the cell division cycle that gives rise to neurons and is thought to influence the type of nerve cell (neuron, astrocyte or radial glia) that results from division of a radial glia cell. A prolonged G₂/M phase was also observed in basal progenitor cells. The effects we observed are likely to be mediated by MOR activation by morphine as a mice lacking a function MOR (MOR KO) did not show an elongation of G₂/M phase. Although morphine exposure altered the duration of the cell

cycle for progenitor cells in the embryonic telencephalon, it did not affect whether the progenitors remained proliferative and re-entered S phase, or whether they exited the cell cycle and became quiescent. In addition, morphine treatment did not change the proportion of basal to apical mitoses. These findings indicate that opioid signalling plays a role in cell cycle progression of both radial glia and basal progenitor cells *in vivo* in the developing cerebral cortex.

Regulation of susceptibility to autoimmunity by interleukin-4 receptor α (IL-4R α)

AC La Flamme¹, D Kenwright², and P Keating¹

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Multiple sclerosis (MS) is a debilitating neurological disease caused by the development of an autoimmune response to normal proteins in the central nervous system (CNS). Current immunological beliefs hold that pro-inflammatory, T helper cell type 1 (Th1) responses promote autoimmune diseases like MS; whereas, anti-inflammatory, Th2 responses suppress disease. The studies supported by this grant investigated how a receptor of Th2 signalling (interleukin-4 receptor α ; IL-4R α) alters macrophage (M ϕ) function to prevent damage in the CNS by self-reactive T cells. We found that contrary to the current belief that Th2 responses suppress EAE, these responses are actually disease-inducing in the Balb/c mouse strain. Furthermore, M ϕ appear to be critical to this process.

Our laboratory investigated the mechanism by which Th2 responses promote disease and found that IFN- γ production by cells from the draining lymph node peaked earlier in IL-4R α ^{-/-} compared to Balb/c animals and suggests that high levels of IFN- γ early in disease may initiate a down-regulatory immune pathway that resolves the self-reactive response before clinical disease manifests. Our studies suggest that in Balb/c mice, Th2 cytokines, signalling through IL-4R α , may regulate this process. Indeed, we found that altering the Th1-inducing stimulus changed the susceptibility of mice to development of disease such that with a lower Th1 stimulus the susceptibility of IL-4R α -deficient animals increased and vice versa. Additionally, we examined whether regulatory T (Tregs) cells were involved in the regulation of susceptibility but did not find any significant correlation between Treg numbers and disease incidence or severity. These studies support the belief that increased pro-inflammatory responses in the absence of IL-4R α signalling induces a down-regulatory pathway that resolves the autoreactive response before overt disease occurs.

Using mice in which the capability to respond to IL-4 and IL-13, through IL-4R α , has been specifically eliminated from M ϕ but not other cells in the mouse, moIL-4R α ^{-/-}, we found that it is the ability of M ϕ to respond to these Th2 cytokines that promotes disease. Compared to their littermate controls,

these mice can produce similar Th2 responses. Therefore, the disease regulating effects are mediated by IL-4/IL-13-regulated M ϕ effector functions. We examined the involvement of nitric oxide synthase-2 and indoleamine 2,3-dioxygenase in M ϕ -mediated T cell inhibition. While these two enzymes have been shown to be effective at inhibiting T cell function in other systems, they do not appear to be the main mechanism by which M ϕ are preventing the development of EAE in Balb/c mice.

Through identifying factors that suppress disease progression in our unique mouse model of MS, we have expanded our understanding of the pathological progression of MS in the human population. Because individuals and genetically distinct mice (i.e. different inbred strains) manifest different MS disease patterns (i.e. relapsing-remitting, primary progressive), our novel finding that Th2 responses promote disease in Balb/c mice, suggests that the immune pathways causing autoimmune-mediated damage may differ between individuals. Thus this enhancement our understanding of the pathways involved in regulating MS development will help in the development of alternative therapeutic strategies to provide effective treatment of patients who do not respond to currently available therapies.

The Cellular Effects of Nicotine and Smoking – Investigating New Cellular Targets for Cessation Therapy

BM Kivell and P Truman

School of Biological Sciences, Environmental Scientific Research

Smoking is one of the leading causes of preventable illness in the world. The Ministry of Health estimates that over 4300 deaths in New Zealand each year are attributed to cigarette smoking. Current cessation treatments based on nicotine replacement therapy are ineffective, with a success rate of less than 20% after 1 year. Smoking releases neurotransmitters such as dopamine and serotonin. Dopamine activates the brains reward system, whereas serotonin acts as a mood elevator. Monoamine transporters function to remove these neurotransmitters from the synapse where they are active, thus terminating the actions of neurotransmitters.

Very few studies have investigated the effects of smoking on the monoamine transporters. In this study we are investigating the effects of smoking and nicotine on the function of monoamine transporters. Using real-time PCR MSc Student Kirsty Danielson has measured the mRNA levels of the serotonin (SERT), dopamine (DAT), and nor-epinephrine (NET) monoamine transporters after chronic exposure to nicotine, and total particulate matter (TPM) from cigarette smoke in various regions of the brain. Results have shown that there are no changes in mRNA expression levels of NET in the locus coeruleus between control and nicotine treated rats (n=5), however, there is a trend towards increased expression in TPM treated animals (n=2). No Changes in SERT expression levels are seen in the Dorsal Raphe between control, TPM and nicotine treated groups. However, we have seen some interesting trends in DAT mRNA expression levels. In the substantia nigra nicotine mRNA levels decreased compared to control and TPM treated

animals; whereas in the ventral tegmental area of the brain TPM caused an increase in DAT mRNA expression (n=2-6). More replicates are currently being analysed and will need to be included before any significant conclusions can be drawn. These results show that the monoamine transporters expression may be altered on exposure to nicotine and TPM. Interestingly there may be differences between nicotine and other components of cigarette smoke. This is important as traditional cessation treatments focus on nicotine, but other components of cigarette smoke may have addictive properties and regulate expression levels of proteins controlling neurotransmitter levels.

The function of monoamine transporters in isolated tissue and in cells has been established in our laboratory using a neurochemical technique called Rotating Disk Electrode Voltammetry (RDEV). Hons student Bridget Simonson has characterised this technique and has measured the uptake kinetics for monoamine transporters in cultured cells and in tissue from control animals. Experiments on nicotine and TPM treated animals will be conducted from September. This technique will allow us to study, for the first time the effects of nicotine and TPM on monoamine transporter function.

Wakefield Hospital

Comparison of the Portal Vein and Peripheral Blood Biochemistries of Diabetic and Non-diabetic Patients over the First Six Days Following Gastric Bypass Surgery

M Hayes
Gastroenterology Research Institute.

There is evidence that the resolution of type 2 diabetes post gastric bypass surgery (GBS) may be due to signalling from those sections of the gut isolated from food following gastric bypass surgery. Our previous studies and evidence from selective knockout of the insulin receptor in mice lead us to believe that insulin resistance in the liver (rather than in muscle or fat) is central to the development of type 2 diabetes. The two structures are directly connected by the portal vein and signals from the gut are likely to pass via this vessel. It is well known that dipeptidyl peptidase-4 (DPP-IV) rapidly degrades a range of signal peptides derived from the gut (some of which have a half life of only 30 sec). Gut peptides generally have very short half lives and are diluted by systemic blood following passage through the liver such that the quantity detectable in peripheral blood samples may be a very small fraction of what is produced by the gut. To investigate gut derived peptides it is desirable to collect blood before dilution and degradation and the best site for this is the portal vein. Collection into tubes which contain a DPP-IV inhibitor will also prevent further degradation of many of these peptides. In order to investigate these peptides it is also necessary to deplete the major proteins in plasma as these obscure differences between samples in proteomic techniques such as 2D gel electrophoresis.

This grant in aid has enabled the purchase of Becton Dickinson P700 DPPIV inhibitor tubes to collect blood samples from the hepatic portal vein of patients undergoing gastric bypass surgery. It has also enabled the purchase of a Proteome Lab IgY-12 SC proteome partitioning kit to deplete the 12 most abundant proteins from plasma samples collected from the portal vein. We have collected portal blood to DPPIV tubes from 28 patients, 20 of whom have both pre and post operative serum samples (it is not always possible to get samples from the 6 day post operative time point). We have now used the depletion kit on 12 samples and will shortly be conducting 2D gel electrophoresis on these samples. The studies are ongoing and have the potential to cast light on the fundamental cause of insulin resistance and type 2 diabetes.

Is the Improvement in Insulin Resistance after Gastric Bypass due to Reduced Energy Intake?

RS Stubbs and J Krebs
Gastroenterology Research Institute

Most type 2 diabetics who undertake gastric bypass surgery (GBS) experience a rapid resolution of their diabetes within 6 days of the operation. Weight loss is not likely to be a contributor to this resolution as it occurs well before any significant weight loss has occurred. The diabetic patients no longer require medication and their insulin resistance as measured by the homeostasis model assessment (HOMA), returns to the near normal range. This study was conducted to ascertain whether reduction in caloric intake was a major contributor to the observed effect and to provide a more robust measure of insulin resistance in the patients.

This research grant funded studies into the effects of a 6 day very low calorie diet (VLCD) on insulin resistance and non-esterified fatty acids and compared these to the effect of gastric bypass on insulin resistance in the same patients. Insulin resistance was measured by HOMA and intravenous insulin tolerance test (ITT) and these were done on the before commencing the diet and 6 days later as well as on the morning of surgery and 6 days later. Blood samples taken for the ITT were also used for determination of the NEFA values. A total of 27 patients were included in the study with 9 undertaking the VLCD, 14 with preoperative ITT and 21 with post operative ITT.

Results from this study showed that the effect of VLCD on insulin resistance as measured by HOMA was not as significant as that produced by the operation over the same time period nor were the patients able to discontinue their medication. While reduced caloric intake may contribute to the resolution of diabetes seen post surgery, it is not the sole reason for the resolution. ITT results produced a quandary since these measurements indicated that the level of insulin resistance increased post surgery yet the patients no longer required medication and their fasting insulin and glucose levels had fallen to within normal range and their HOMA values had reduced. These conflicting results (between ITT and HOMA) may be due to the differences in what is being measured in these two methods. In HOMA,

the calculation is based on the interaction between the liver and the islet of Langerhans in the pancreas and is more a measure of insulin resistance at the liver than of total body insulin resistance. During an ITT, insulin is injected intravenously and the fall in blood glucose level in response to the insulin bolus is monitored. This provides a measure of whole body insulin resistance. We interpret these results to suggest that peripheral insulin resistance (muscle and fat) is less important to the resolution of type 2 diabetes than central insulin resistance in the liver. The findings are new and have very important implications for the direction of further research into the fundamental cause of type 2 diabetes. Because of the unexpected results obtained in the early phases of the proposed study, it was discontinued before the funds provided by the WMRF were fully expended and permission was obtained from the foundation to use the balance of the grant on other diabetes related work.

One feature of the resolution of diabetes following GBS is that the effect is not universal. We see it in around 85% of diabetics following surgery. Developing a model to predict which patients are likely to have resolution of their diabetes following surgery would be informative to the patient and identification of the most important factors might provide further leads to the cause of this reversal. We have used the balance of the funds available to further this area of work. In particular, the funds were used to complete a number of assays for insulin, glucose and c-peptide on serum samples held within the Research Institute. The data obtained, coupled with other data already held by us, were used to create a model to predict resolution of diabetes after gastric bypass. Data (37 parameters) for 127 diabetic patients were analysed to develop a model which predicts whether a patient will or will not resolve their diabetes and is correct 87% of the time.

Laser Microdissection Microscope Facility

R Stubbs and K Hood
Wakefield Gastroenterology Research Institute

Laser microdissection (LMD) is a powerful technique that allows discrete regions of tissue or cells to be isolated from bulk tissue sections for analysis. The technique is particularly powerful for analysis of tumour tissue, since tumours frequently contain non-cancerous cells such as immune, blood or stromal cells and nearby regions of normal tissue. Pure populations of cancer cells obtained by LMD can be used for analysis of genetic abnormalities, gene expression and proteins.

Previously, use of LMD required travel to either Dunedin or Auckland to use LMD facilities. This increased research costs, and slowed research progress, and in some cases precluded work being undertaken. In March 2008, The Wellington Medical Research Foundation provided a grant in aid of \$22,310 towards the purchase of a Leica LMD600 to establish a LMD Facility in Wellington, located at The Wakefield Gastroenterology Research Institute. The LMD Facility also received financial support from New Zealand Lotteries, the Wellington Division of the Cancer Society, and The Wakefield Clinic.

Modern LMD is undertaken by directly mounting fresh or archival tissue sections onto slides containing a thin polyethylene tetrathalate (PET) membrane. The tissue is then fixed, histologically stained and visualized using the LMD microscope which is equipped with a digital camera. Computer software is then used to draw around the cells required for capture, and a laser then cuts through both the PET membrane and tissue, which is removed to a separate tube for analysis (Figure 1). Many types of analysis have been undertaken using cells obtained by LMD including genetic analyses (comparative genomic hybridization and genechip arrays) RNA expression (real-time PCR and microarrays) and proteomics (SELDI and 2D-DIGE).



Figure 1: A 20 μm cryosection of a primary colorectal tumour was fixed in ethanol and stained with toluidine blue. The tissue is first visualized by microscopy (A), and the region of interest (in this case an invasive region into muscle) was marked using computer software (B). A laser automatically cuts the identified border, and the cells inside are removed for analysis (C).

The LMD Facility will be used by a number of biomedical research groups within the Wellington region. Some of these are summarized below.

Researchers at the Wakefield Gastroenterology Research Institute, Department of Pathology Otago University, and The Centre for BioDiscovery and School of Biological Sciences, Victoria University of Wellington, are using LMD to investigate the molecular mechanisms underlying colorectal cancer metastasis. In particular, whether primary colorectal tumours contain a population of metastatic precursor cells at the invasive front that have a characteristic protein profile similar to that of metastases arising from the primary tumour. LMD has been used to isolate distinct populations of tumour cells from primary colorectal tumours, which are then profiled using proteomics to identify important prognostic information about patient tumours.

Professor Brett Delahunt, Dr David Lamb and colleagues from the Department of Pathology & Molecular Medicine, Wellington School of Medicine, University of Otago, will use LMD to examine novel biomarkers in prostate cancer specimens. Prostate needle-biopsy specimens obtained from patients from the TROG 96.01 prostate cancer trial will be used to establish the differences in the molecular make-up between tumour grades within individual patients. LMD will be used to extract DNA from normal and cancerous prostate tissue. The DNA will be analysed with clinical data to determine if there is a correlation between patterns of genetic changes,

tumour grades, dynamics of prostate specific antigen (PSA response signatures-PRS) and biological behaviour of tumours in individual patients to allow better prediction of patient outcome.

Dr Darren Day and colleagues from the School of Biological Sciences, Victoria University of Wellington will use LMD to investigate molecular changes associated with haemangioma progression. Haemangioma is a primary tumour of the vasculature and is the most common tumour of infancy and can cause considerable disfigurement, particularly facial tumours. These tumours are characterized by excessive angiogenesis primarily caused by proliferation of endothelial cells although there is also an accumulation of other cell types such as mast cells, pericytes and macrophages. LMD will be used to isolate putative mesenchymal stem cells from these tumours and to characterize the protein and mRNA expression profiles of these cells from a bank of frozen tissue samples. This will help to determine whether the survival factors identified from previous *in vitro* analyses are also being produced *in vivo* and to identify regulatory components controlling tumour growth and regression.

Professor Ken McNatty and colleagues from the School of Biological Sciences, Victoria University of Wellington will use LMD to investigate reproduction-associated gene expression changes. Previous data indicate that ovulation may be influenced by significant cross-talk between the developing oocyte and the surrounding tissue. LMD will be used to isolate these specific cell-types from reproductive tissues. The relative level of expression of genes involved in reproduction will then be measured using quantitative RT-PCR in these different tissue types to gain a better understanding of their role in the control of ovulation.

Research Grade Epifluorescence Microscope and Image Capture and Analysis Capability for Gastrointestinal Disease Research

K Hood, M Hayes and R Stubbs
Wakefield Gastroenterology Centre and Research Institute

The Wakefield Gastroenterology Research Institute has two research programmes: (a) the Wakefield Colorectal Cancer Research Group is investigating alterations in protein localization and abundance within tumours that are associated with colorectal cancer metastasis and (b) the Wakefield Obesity/Type 2 Diabetes Research Group is undertaking analysis of gene expression associated with insulin resistance, both at the mRNA and protein level. Both projects required the use of a microscope with epifluorescence and image capture and analysis capabilities.

The microscope is an integral part of the laboratory and is used daily. Researchers working on the colorectal cancer research project have used the microscope extensively to analyse protein changes occurring during colorectal cancer progression measured using immunohistochemical staining. This has included analysis of TRAIL receptors, validation of candidate biomarkers

identified from proteomic studies, analysis of putative cancer initiating cells within tumour, in addition to standard haematoxylin and eosin histopathological assessment of tumour samples.

Researchers working on the obesity and Type 2 diabetes project have used the microscope to examine binding of fluorescently labeled insulin to the insulin receptor in various tissues and to examine whether insulin peptide could be detected in duodenal tissue. This work derives from the finding that messenger RNA for insulin was found in total RNA extracts from a number of duodenal samples and from commercially obtained human duodenal RNA. We will also be using *in situ* hybridization to examine which cell types in the duodenum might be expressing insulin mRNA. This technique uses a fluorescently labelled riboprobe to bind to insulin mRNA which is then visualized using the epifluorescent features of the Nikon microscope.

The microscope takes high resolution photographs, easily and quickly and digital images can be viewed in real-time via the computer screen, reducing eye and neck strain from looking directly down the microscope for long periods. The photographs obtained using the microscope have been used in 5 oral presentations, 3 poster presentations, and 2 scientific manuscripts accepted for publication since its purchase, and a number of other publications in preparation.

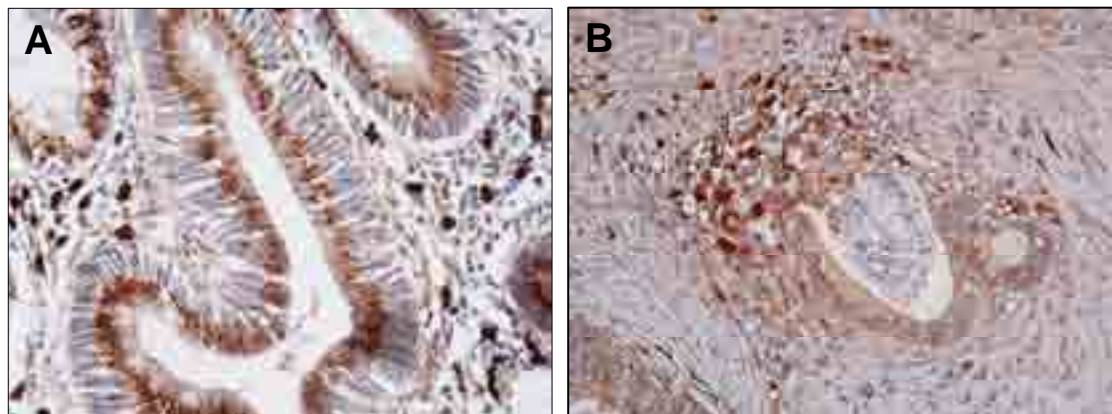


Figure: Photograph of immunohistochemistry taken using the Nikon 80i microscope. Both proteins were visualized using DAB (brown) with haematoxylin (blue) counterstain. **A)** TRAILR-3 localised within subcellular vesicles within primary colorectal tumour cells and **B)** Cathepsin D produced by cells during invasion of the cancer into the bowel wall during colorectal cancer metastasis.

The Role of TRAIL and the TRAILRs in Colorectal Cancer Metastasis

K Hood and R Stubbs

Wakefield Gastroenterology Research Institute

The Wakefield Gastroenterology Research Institute has been interested in identifying genes that prevent the metastatic spread of colon tumours to the liver (metastasis suppressor genes). This research has identified that allelic loss of chromosome 8p occurs in the majority of liver metastases when compared with the primary colorectal tumours from which they had spread. This finding suggested there may be one or more metastasis suppressor genes on chromosome 8p. This finding led to further work, funded by the Wellington Medical Research Foundation, in which we identified the INF α -related apoptosis-inducing ligand receptors (TRAILRs) as molecules with a potential role in CRC metastasis.

There are currently five known receptors for TRAIL. The two pro-apoptotic TRAIL receptors, TRAILR-1 and TRAILR-2 contain extracellular ligand binding domains and intracellular signal transduction domains, including death domains. Ligation of TRAIL to these receptors leads to induction of a series of events which lead to cell death, a process known as apoptosis. Loss of these receptors might therefore allow tumour cells to escape this process and survive when they arrive in the liver thereby allowing metastasis formation. TRAILR-3 and TRAILR-4 have lost this ability to lead to apoptosis and it has been speculated (although is controversial) that these two receptors act as “decoy receptors” which sequester TRAIL away from the pro-apoptotic TRAILRs, thereby preventing TRAIL-induced apoptosis. An additional receptor, osteoprotegerin (OPG), has been shown to bind TRAIL. OPG is a soluble receptor that has been proposed to function like a decoy receptor, although OPG has low binding affinity for TRAIL *in vitro*.

The first objective for the project was to investigate the abundance and distribution of the four TRAILRs in primary colorectal tumours which had not metastasized, in those that had metastasised and in liver metastases. Staining of the four TRAIL receptors, TRAIL and OPG has been completed in 60 colorectal tumour samples. The staining intensity and the percentage of positively stained cells in each tumour has been scored. Clinicopathological data, including tumour stage, location, grade, disease recurrence, etc was collated for each sample and analysed for the staining intensity to determine whether levels of the TRAILRs or TRAIL correlated with clinicopathological data. All four TRAIL receptors had a unique pattern of expression within tumours. TRAILR-1 was absent from stroma, but expressed on the luminal surface of cancer epithelial cells. TRAILR-2 was expressed by both epithelial cancer cells and stroma cells. TRAILR-3 was localized to discrete vesicles located at the apical surface of cancer epithelial cells and TRAILR-4 was expressed equally in the stroma and epithelial cells. OPG was produced predominantly by smooth muscle cells, although weakly by myofibroblasts in the stroma. TRAIL staining was discrete and restricted to epithelial cancer cells. TRAILR-2 had a significantly lower level of expression in liver

metastases compared with the primary colorectal tumour, a finding which may be of relevance to the development of the liver metastases.

Project Grants 2008

The following projects were approved for funding in May 2008 and will be reported on in subsequent Annual Reports of the Foundation.

Darren Day

Victoria University of Wellington

Relating neurochemical changes associated with cannabis use to juvenile learning and memory

The aim of this project is to investigate the neurochemical changes THC exposure has on neurogenesis in the juvenile rat and to explore the interaction of the opioid and cannabinoid systems in Lippocampus following THC exposure. The prevalence of cannabis use and abuse in New Zealand poses a significant public health issue that places the developing minds of teenagers experimenting with cannabis during a critical period of final neuronal maturation at risk. This project aims to better understand the consequences that experimentation with cannabis has on adolescence memory and learning.

Jacqueline Harper

Malaghan Institute of Medical Research

Monocyte phenotypes in gouty inflammation

Gout is a significant public health problem in New Zealand causing a large amount of human suffering. Up to 20% of individuals are unable to manage the disease effectively with the current medical regimes.

Gouty arthritis is an inflammatory condition triggered by the deposition of monosodium urate crystals (MSU) in the joints. By understanding how monocyte activity is involved in the inflammatory response to MSU it will be possible to identify cellular targets and assay systems for development therapies.

Kylie Hood

Wakefield Gastroenterology Research Institute

Characterization of the Invasive Front of Metastatic Colorectal Tumours

Accurate identification of the metastatic precursor population in tumours is vital to the development of clinically useful new molecular diagnostics and drug targets aimed at preventing or treating metastasis, since these need to target the relevant population of cells that is responsible for the aggressive disease progression. The project involves the use of the latest technological advancements in proteomics to address an important clinical problem.

Isabelle Hoong

Massey University

SPIRIT Study: Beneficial health effects for Polynesians with type 2 diabetes?

The incidence of type 2 diabetes and obesity is rapidly escalating in New Zealand, especially in the Polynesian population. This study aims to investigate the effect of progressive resistance training exercise on obesity, glycaemic control and insulin resistance in obese and diabetic Polynesians residing in the greater Wellington region.

Anne La Flamme

Victoria University of Wellington

Diagnostic markers of hepatosplenic schistosomiasis

Schistosomiasis is a parasitic disease that afflicts more than 200 million people worldwide. This project has two goals: to determine the impact of schistosomiasis on protein expression in the liver and to develop assays to detect marker proteins in serum to enable early detection of infected individuals at high risk of developing severe hepatosplenic disease.

Franca Ronchese

Malaghan Institute of Medical Research

Adoptive T cell transfer therapy of cancer

Adoptive cell transfer (ACT) is a method of cancer immunotherapy that involves isolating tumour specific CD8 + T cells from a patient's blood or tumour tissue, activating and expanding these cells in vitro, and infusing them back into the patient. The project will characterise the specific elements required for the generation of tumour-specific T cells that are capable of rejecting tumour challenge in vitro.

By determining the importance of the relationship between the antigen presenting cells and T cells during activation and investigation of the in vivo requirements for survival and anti-tumour function, this research might be able to further improve the success rate of ACT and establish this promising therapeutic strategy as a viable mainstream therapy of cancer.

Shieak Tzeng

University of Otago, Wellington

Different effects of anaesthetic agents on respiratory sinus arrhythmia (RSA) in the rat

The research group for this project has a special interest in cardiovascular autonomic research, and has focused on the role of the parasympathetic nervous system (PNS) on mediating respiratory sinus arrhythmia, which usually manifests as an increase and decrease in heart rate during inspiration and expiration respectively.

The outcomes of this study will contribute to the assessment of an anaesthetised rat model more suitable for future RSA research where protocols cannot be applied to human volunteers.

Angela Gruber

Diabetes Unit, Wellington Hospital

The impact of a lifestyle modification programme

This project aims to investigate the effects of low impact exercise classes on diabetes control, future exercise and quality of life in a group of overweight and obese patients with diabetes and poor exercise tolerance.

Peter Ferguson

Victoria University of Wellington

Iron Carbide nanoparticles as magnetic resonance (MRI) contrast agents

The incidence and mortality rates of malignant melanoma have more than double in New Zealand over the past three decades, and remain some of the highest in the world. Early diagnosis with accurate staging may reduce morbidity and prolong survival.

This project has the potential to develop novel magnetic resonance contrast agents with greater efficacy and safety than other agents available on the market. Effectively deployed, these agents improve the ability of MRI to detect cancer in its earlier and treatable stages.

Melanie-Jane McConnell

Malaghan Institute of Medical Research

Sirtuins, Stress and Survival: A problem in anti-tumour therapy

The fundamental aspect of cancer cell survival is the ability of cancer cells to change metabolic pathways to cope with cellular stress. This can impact on the effectiveness of anti-tumour compounds induction of a stress response by a compound can enhance survival mechanisms resulting in less tumour cell killing.

Understanding the survival mechanisms is key to designing effective anti-tumour therapeutics.

Travel Grants

13th Lorne Proteomics Symposium and the 20th Lorne Cancer Conference, Australia, February 2008

A Wilmes

I would like to thank the Wellington Medical Research Foundation for their kind support in the form of a travel grant to attend the 13th Lorne Proteomics Symposium and the 20th Lorne Cancer Conference in Australia in February 2008.

At the Proteomics conference I presented “Comparing the cellular responses to peloruside A and paclitaxel, two microtubule stabilizing drugs, using DIGE proteomics”. In my talk, I summarised the proteomics results of my PhD research which compared the effects of peloruside A with the effects of a widely used anti-cancer drug paclitaxel in human leukaemia cells (HL-60). Briefly this study found a number of similarities between both compounds, as expected for two drugs that share a common mechanism of action. However, most of the protein changes identified were unique for each drug, highlighting the differences between the two drugs. Our results were also analysed using network pathway analysis, and even though both drugs gave similar networks, each network contained unique proteins. In addition peloruside changed fewer proteins compared to paclitaxel at concentrations that arrested a similar percentage of cells in G2/M phase of the cell cycle. Overall our results support the continued development of peloruside as an anti-cancer agent.

The talk was very well received and I had the opportunity to talk to a number of other researchers in the proteomic field about my research. In addition it was fantastic to listen to all the other proteomic talks as it gave me a number of new ideas as well as useful information about new proteomic techniques.

At the Cancer conference I presented a poster on “Deciphering the binding site of peloruside A using peloruside-resistant cells”. The work presented on this poster used a resistant cell line to help identify the unknown binding site of peloruside on the tubulin molecule. This resistant cell line had a mutation within tubulin that led to a change from a hydrophobic to hydrophilic amino acid. The cells were only resistant to peloruside but not to any other microtubule targeting drug that was tested, suggesting the mutated amino acid might correlate with the unique binding site of peloruside on the microtubule. Again this conference was a great opportunity to make contact with other researchers in the field as well as to get new ideas and directions for my research.

2007 New Zealand Medical Sciences Congress, Queenstown

P Sin

I would like to thank the WMRF for the travel grant for my attendance to the New Zealand Medical Sciences Congress in Queenstown last November.

For my first conference attendance, I found it to be a thoroughly enjoyable and informative experience. It was interesting seeing the range of research going on in my field and meeting various researchers. I have also started to build useful contacts with other researchers. One of the very helpful things that happened was a presentation that exhibited useful technology that we may well incorporate in our research.

My presentation *Respiratory sinus arrhythmia examined by the RSA curve* was well received and was a good learning experience. I look forward to doing more in the future.

14th Annual Meeting of the Society for Free Radical Biology and Medicine (SFRBM) in Washington DC, USA, November 2007

A Tan

I am very grateful for the financial support from the WMRF that enabled me to attend the 14th meeting of SFRBM in Washington DC, USA, November 14-18, 2007. This also provided me a golden opportunity to present new research findings, to receive feedback from international experts in the field and to meet other researchers from different countries for research exchange and collaboration.

The SFRBM 2007 meeting attracted over 700 scientists, researchers and clinicians from around the world to Washington DC for five days of exciting scientific presentations. The format of the meeting included a full day pre-meeting workshop on protein oxidation and modification-mechanisms, measurement and biological consequences. The schedule for the following four days included sunrise free radical school, plenary lectures, oral presentations of selected abstracts and poster symposia. The sunrise free radical school was interesting because it provided a detailed overview of the basic concepts of free radical chemistry and biology and targeted towards students, fellows and those wishing to learn about new areas.

The conference covered a broad range of scientific topics, ranging from plant to animal studies and from basic to clinical and industrial research. Besides the keynote lectures, there were numerous interesting sessions on a wide range of free radical biology topics.

I presented a poster entitled "Cellular trans-plasma membrane electron transport mediates redox cycling of the two-electron reducing quinone, DMNQ, via NQO1: a sensitive cellular microplasm assay for NQO1". This work is the first to indicate that plasma membrane electron transport (PMET) plays a critical role in quinone oxidative cytotoxicity and this toxicity process is mediated via the phase II enzyme NQO1. In addition, we have also developed

a new simple and sensitive cellular microplate assay for measuring NQ01 activity.

2007 New Zealand Medical Sciences Congress, Queenstown

SYC Tzeng

As an emerging researcher I very much appreciated the financial support from the WMRF towards costs to attend the conference. Attendance has been very beneficial both in disseminating my research, as well as building new contacts, and consolidating existing relations with researchers from other research institutions.

I felt this year's conference went particularly well. In the Respiratory Physiology section our work from the Physiological Rhythms Unit generated much debate and interest. I was able to establish potential collaborative efforts with Physiologists based in Massey University and Otago researchers based in Dunedin. As a direct result of these contacts we are now in the process of initiating some collaborative research for 2008.

Ongoing attendance has also been helpful in establishing a presence for our small research group. As a unit within a clinical school of medicine we often find ourselves working in isolation from scientists working in research institutes or on major university campuses. Presenting research regularly at meetings, such as the Medical Science Congress, enables our laboratory to stay connected to the New Zealand scientific community.

37th ASM Australasian Society for Immunology

J Qin

The 37th Australasian Society for Immunology conference was held in Sydney, Australia on 2-6 December 2007. I am grateful for the financial support provided by the Wellington Medical Research Foundation to allow me to attend this conference. At the conference I was able to present a poster on dendritic cells maturation and how that affects cross-presentation of protein antigens and how it influences dendritic cell function and survival.

The conference provided a great opportunity to present my work outside my institute to international researchers. My poster detailed the work in our laboratory investigating how the maturation level of dendritic cells affects their ability to cross-present antigen and how that in turn affects its ability to generate an immune response against the antigen. It was positive to be able to present and discuss the work with many different international researchers. As a researcher in the field of immunology the conference has to a great extent expanded my understanding of the many areas of current immunological research. It has also provided me with a clear view of the future of the field, and where I should focus my future research. I again thank the foundation for providing me with the opportunity to attend this conference.

Society for Neuroscience Annual Meeting, San Diego, California

A Lewis

My attendance at Neuroscience 2007 allowed me to present a poster detailing some of the results and experiments I have completed as part of my PhD research. I presented my poster entitled Denicotinised tobacco extract inhibits monoamine enzyme activity in SH-SY5Y cells on Sunday 4 November, in a poster session for research on different models of nicotine independence. Briefly, the poster detailed the research I have completed into the effects of standard and denicotinised cigarette extracts on the activity and gene expression of Monoamine Oxidase A and Monoamine Oxidase B enzymes in cultured cells.

Utilising the MAO sensitive fluorescent substrate, Kynuramine, MAO enzyme activity was measured in SH-SY5Y cells treated over several days with nicotine, tobacco total particulate matter (TPM) extract, and denicotinised tobacco TPM extract. It was confirmed that both the standard and denicotinised tobacco extracts inhibited total MAO activity. This inhibition was also observed in purified recombinant MAO-A and MAO-B when exposed to these extracts.

Preliminary quantitative reverse-transcriptase PCR experiments have found no significant decrease in MAO-A or MAO-B gene expression in SH-SY5Y cells, which suggest that the MAO inhibition observed after exposure to tobacco is a result of inhibitory compounds acting on the enzymes directly, and not through reduced MAO gene expression.

My poster was received well, and gained some interest from fellow researchers in the tobacco dependence field from Duke University, Arkansas State University and the University of Toronto (UTSC) in particular. I was able to discuss similarities in the research we were doing with other doctoral students present, and compare the results we were achieving, which I found very valuable. As a result of discussing my experiments I have been able to reassess some anomalous results I had received and dismissed as artefact. Further experiments may show that purified nicotine may inhibit MAO activity through an indirect mechanism, whereas previous research has described no effect on MAO activity by purified nicotine.

Summer Student Research Reports



Audit of Implanted Cardioverter Defibrillators in New Zealand

Praveen De Silva

Implanted Cardioverter Defibrillators (ICDs) still remain the standard of care for those at risk of Sudden Cardiac Death (SCD). While a number of trials have demonstrated the effectiveness of this therapy, no-one has previously examined how effectively ICDs are being used in New Zealand to reduce SCD. This was a retrospective audit of ICD clinical practice in New Zealand from 2000-2006. The audit examined indications and outcomes for ICD patients in NZ.

All patients who received an ICD implant in Wellington and Auckland between 2000-2006 were reviewed. Data collected included patients' ICD implant and testing notes, past medical history, ICD therapy and subsequent all cause hospitalisations and mortality. We examined implantation rates across the study period, the proportion of primary vs secondary implantation, complications during implant, ICD therapy and all cause mortality rates.

571 devices were implanted between the two centres from 2000-2006 with a peak implant rate of 39/million in 2004. Devices were principally implanted for secondary prevention. The proportion of implants for primary prevention did not change significantly over the study period with 46% in 2000 compared to 44% in 2006. No patients died during implantation and complications at implantation remained relatively unchanged at a 10% level. At one year 26% of patients had received therapy for VT or VF and at five year 45% had received therapy. All cause mortality was low with 6% mortality at one year and 12% mortality at five years.

Implantation rates and the use of ICDs for primary prevention remain low by international standards however patients are still receiving a high rate of therapy at one and five years post-implant. There has been no mortality at implant and subsequent all-cause mortality remains low.



The Role of Cognition in Independent Community Walking: Which Variables Predict Gait Speed?

Leigh Halkett

Walking in community environments is highly complex, requiring motor and cognitive flexibility to cope with the demands of challenging terrain and unpredictable events. There is increased interest in measuring gait in 'real world' conditions rather than in laboratory or clinical settings that do not accurately reflect the nature of this complex task.

This preliminary study is part of a larger observational study, of 130 participants, that sets out to test a theoretical framework of community ambulation, and explore cognitive, motor and behavioural characteristics that may be predictive of gait speed. This pilot study was confined to exploring the associations between measurements of cognitive characteristics, including attention, that predict gait speed in independent community walking.

The sample consisted of 28 women and 13 men with a mean age of 76.9 years (± 7.6) who lived and walked independently in the community. Measures of visual selective attention, divided attention and cognitive functioning correlated moderately and significantly with gait speed (*Pearsons $r = 0.35$ to 0.47*). The significant explanatory variables were entered stepwise into a regression model, with gait speed as the dependent variable. Visual selective attention was retained in the model, and explained 22% of the variance in gait speed. This may reflect the important role of vision to adaptive locomotion. Through scanning of immediate and future location, vision guides gait activity and enables advance planning of gait modification and route finding.

Findings from this pilot study suggest that cognition and attention are important attributes of independent community walking. However, the sample was small and the results must be interpreted with caution. A larger sample size will yield confirmatory findings.



Early Vascular Disease In Children With Epilepsy Receiving Anticonvulsants

Ngaire Keenan

Patients with epilepsy have been found to have a four fold risk of cardiovascular disease. This increased risk is due to antiepileptic drug treatment, such as carbamazepine and Valporate, and its effect on homocysteine metabolism, lipids, cholesterol and autonomic functions. Antiepileptic treatment, especially enzyme inducers such as carbamazepine, decrease important cofactors, folate and vitamin B6 (pyridoxal-5-phosphate), increasing total plasma homocyst(e)ine concentrations. Homocysteine, an important risk factor for atherosclerosis causes endothelial dysfunction via oxidative stress. Early development of atherosclerosis is best measured using the ultrasound techniques intima media thickness and flow mediated dilation. Atherosclerosis at its early stages can be prevented to improve future health. Vitamin supplementation of the important cofactors required for homocysteine metabolism is an effective intervention in patients with epilepsy, decreasing total plasma homocyst(e)ine to levels of untreated controls.



Mosquito activity in the Waikanae peri-urban area and around dairy farms on the Manawatu floodplain with implications for human exposures to nuisance and potential arbovirus carrying mosquitoes

J Pizarro

This project examined mosquito activity around Waikanae urban parks and gardens and on dairy farms at three rural locations on the Manawatu-Orua floodplain outside Palmerston North. Both are highly modified landscapes and both were affected by serious flooding in February 2004.

Objectives:

- to sample adult mosquitoes at each location
- to sample standing water for larvae (breeding habitats) & characterise sites

- to undertake laboratory identification & enumeration of adults & larvae
- to review resident & local body comment on mosquito nuisance
- to consider implications for human exposures to nuisance and potential disease carrying mosquitoes & for the mosquito response to flooding events.

Mosquitoes were sampled over the period starting 11 December 2007 and this will be continued to early March 2008 to cover the peak biological season for adult mosquito activity.

We found six species of mosquitoes around Waikanae gardens and parks (urban, some remnant native wetland & swamp forest habitat) and three in the Manawatu rural area (more monocultural, no remnant native vegetation), Table 3. These include two exotic vector species which are, significantly, present at both locations.

The species, as characterised by catch per trap.night, fall into two distinct groupings. These correspond closely to ecological characteristics and also to their biting habits and thus to potential health concern. The two *Culex* species are more generalist in habits and most productive in anthropic habitats, while in New Zealand *Ae notoscriptus* has become widespread though is most abundant in urban and peri-urban habitats. In contrast the native *Cq* species and *Ae antipodeus* which form a separate grouping, are restricted to areas with some remnant native habitat. These seem to be more opportunistic human biters.

It is significant that the farming area has greater abundance of fewer species and that these are known, plus one potential, vectors of human and some animal viruses. Ecologically this finding is consistent with the tendency to short-term monoculture typical of NZ farming systems, with consequent loss of natural regulatory function in low diversity biotic communities. Thus there are opportunities for invasive insects to proliferate, and generally greater numbers of few insect species and these tend to be the more r-selected generalist and opportunist ones. These are also the ones more likely to cause concern as pests, or carriers of pathogens.

The order of mosquito numbers represents low abundance (mean catch < 50/trap.night) and can be considered a background rate, given the 'La Nina' weather regime with unusually warm and dry conditions on the western side of the North Island. Fortuitously it has also provided a means to locate 'refuges' of mosquito persistence, which would otherwise be obscured by more widespread adult activity.

Since the dry summer to date has greatly restricted mosquito activity, there has been minimal reporting of mosquito nuisance. However, there is clear potential for a significant response in mosquito productivity to a favourable combination of seasonal timing and weather, as was demonstrated in the Waikanae area after serious flooding in 2004.



Policymaking for Smokefree Outdoor Public Places in the Wellington Region

Sharon Tay

To explore the knowledge and perceptions of councillors in the Wellington region about outdoor smokefree policies, in order to identify themes and policy implications.

Global advancements in health awareness have spurred the introduction of smokefree policies. Apart from indoor bans, several jurisdictions have imposed outdoor bans on smoking in some places. Several New Zealand district councils use educational policies, rather than legislation, to promote smokefree outdoor places. The development of such policies does not appear to have been studied within New Zealand or overseas.

Twenty-one councillors were given in-person, semi-structured interviews during December 2007.

Most of the interviewees agreed that outdoor smoking would affect role modelling (86%) and create litter (76%), whereas other impacts including harm to others' health attracted less agreement. Many interviewees acknowledged a lack of information about such impacts, highlighting the need for dissemination of demonstrable evidence. There was very limited knowledge about existing outdoor smokefree policies elsewhere (38%), and limited support for both such policies (52%) and council intervention (52%). Most councillors identified a range of potential obstacles to effective policy implementation. Some suggested that such obstacles could be overcome by education and advocacy to increase awareness and public support. Trials of outdoor smokefree policies and outcome assessments of existing policies in New Zealand or overseas might be useful.

A multifaceted approach to the development of outdoor smokefree policies appears to be needed, including public education and the demonstration of public support.



Exploring changing prescription charges – a survey

Emily-Jane Willmot

The price of prescriptions is an important determinant in access to health care, New Zealand has a complex pricing system for patient prescriptions which involves multiple patient subsidy levels and differential costs to patients dependent on prescriber. In July 2007 prescription prices were decreased from \$15 to \$3 when prescribed by Primary Health Organisations (PHOs).

This research aims to determine the level of knowledge and implications of recent changes to prescription prices in New Zealand that occurred in July 2007.

Two face-to-face surveys were conducted involving pharmacists (n=20) and the community (n=80).

In the community survey, 73.8% were unaware of the price changes and 67.5% were unaware that the cost varied dependent on whom the prescriber was. 8.75% of respondents cited cost as the reason for filling a prescription in the last 6 months. Having been informed of the prescription price change 28% stated that this change would increase the likelihood they would see a doctor when they are ill to get a prescription. Pharmacists perceived that this change had decreased their profit, and 20% reported patients who took specialist prescriptions to their GP to have it rewritten to obtain the PHO price.

This study showed that the majority of community participants were not aware of the price change, and this lack of knowledge could be a significant barrier to healthcare. The price discrepancy between prescription providers is difficult to explain to the public and introduces price inequalities. It is critical that the inequalities in access to cheaper medications are reviewed and the complex pricing system is simplified to eliminate disparities between providers. An appropriate media campaign should be undertaken to inform the public of these changes.

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