## DIABETES AND OBESITY PROGRAM

**James Group (Cell Biology)**
**Dr. David James (d.james@garvan.org.au)**
**Ph: (02) 9295 8210**

### Project 1: Novel actions of insulin and other growth factors

Using mass spectrometry we have recently discovered several novel insulin regulated phosphoproteins that involve, regulation of the cytoskeleton, RNA processing, thyroid metabolism and vesicular transport. These represent exciting new areas for investigation using molecular and cellular techniques as well Genetrap knock out mice.

### Project 2: Regulation of vesicle transport

Rab proteins play an intimate role in regulating the traffic of membrane proteins in the cell. We are currently actively engaged in several exciting projects using total internal reflection microscopy, x-ray crystallography as well as mathematical modelling to unravel the mechanism of these proteins in the living cell. Again using mass spectrometry we have identified a number of novel effectors and in collaboration with researchers in Genentech at San Francisco we are actively pursuing their function.

### Project 3: Insulin resistance

Insulin resistance is often considered the major defect leading to Type 2 diabetes. We have recently published exciting new findings concerning the mechanism of insulin resistance. These findings have led us to oxidative stress as a major node of insulin resistance and so we are diverting considerable effort to the mitochondrial bioenergetic system in order to understand how this organelle communicates with other essential functions in the mammalian cell.

### Recent Publications

Bai, L., Wang, Y., Fan, J., Chen, Y., Ji, W., Qu, A., Xu, P., James, D.E., Xu, T. Dissecting multiple steps of GLUT4 trafficking and identifying the sites of insulin action. *Cell Metabolism* 5, 47-57, 2007 IF 17.1

Hu, S-H., Latham, C.F., Gee, C.L., James, D.E., Martin, J.L., Structure of the Muc18c/Syntaxin4 N-peptide complex defines universal features of the N-peptide binding mode of Sec1/Munc18 proteins. *Proc. Natl. Acad. Sci. U.S.A*. 104, 8773-8778, 2007. IF 9.6

Ng Y, Ramm G, Lopez JA, James DE. Rapid activation of Akt2 is sufficient to stimulate GLUT4 translocation in 3T3-L1 adipocytes. *Cell Metab* 7:348-56, 2008. IF 17.1

Tan M-J, Ye J-M, Hohnen-Behrens C, Ke C-Q, Tang C-P, Rowland A, Turner N, Chen T, Weiss H-C, Gesing E-R, James DE, Ye Y. Anti-diabetic activities of newly-identified triterpenoids from bitter melon associated with activation of the AMPK pathway. *Chem. Biol*. 15:263-73, 2008. IF 5.7

Hoehn KL, Hohnen-Behrens C, Cederberg A, Wu LE, Turner N, Yuasa T, Ebina Y, James DE. IRS1-independent defects define major nodes of insulin resistance. *Cell Metab*. 7:421-33, 2008. IF 17.1

Hocking SL, Chisholm DJ, James DE. Studies of regional adipose transplantation reveal a unique and beneficial interaction between subcutaneous adipose tissue and the intra-abdominal compartment. *Diabetologia*. 51:900-2, 2008. IF Citations 2

**Phospholipid Biology Group**
**Dr. William (Will) E. Hughes (w.hughes@garvan.org.au)**
**Ph: (02) 9295 8216**

Coordinated trafficking of molecules within cells is a critical process. We have been studying the regulation of the vesicle based trafficking fundamental to the secretion of insulin and translocation of glucose transporter (GLUT4) from intracellular storage compartments to the plasma membrane. We use cell biological (particularly live cell microscopy) and biochemical approaches to identify *where*, *when* and *why* particular phospholipids are produced (or destroyed) to regulate the exocytosis of insulin and GLUT4 processes. We are interested to discover how these processes may be disrupted in diseases, particularly diabetes and cancer. Projects in the laboratory currently include:

**Project 1: Regulation of vesicle/plasma membrane fusion**
The enzyme phospholipase D (PLD) produces the phospholipid phosphatidic acid. This phospholipid can act as a signalling molecule but seems to play a direct role in mediating membrane fusion events. We are studying what role the enzyme plays in regulating insulin exocytosis from pancreatic-beta cells.

**Project 2: The role of phosphatidylinositol 3-kinases (PI3-K) in signal transduction**
The phospholipid PtdIns3P appears to play an indirect but essential role in regulating a final step in the insulin stimulated exocytosis of glucose transporter (GLUT4). We are aiming to identify *where*, *when* and *how* this phospholipid performs it's role in muscle cells and adipocytes.

**Recent Publications**

The Role of Phosphoinositide 3-Kinase C2$\alpha$ in Insulin Signaling (2007).
Falasca, M., Hughes, W. E**.**, Dominguez, V., Sala, G., Fostira, F., Fang, M.Q., Cazzolli, R., Shepherd, P.R., James, D. E., and T. Maffucci, *Journal of Biological Chemistry* 282, 28226-28236.

Phospholipid signalling through phospholipase D and phosphatidic acid (2006).
Cazzolli, R., Shemon, A.N., Fang, M.Q. and Hughes W.E. *IUBMB Life*. 58, 458-461.

PLD1 regulates secretagogue-stimulated insulin release in pancreatic $\beta$-cells (2004).
Hughes, W. E., Elgundi, Z., Huang, P., Frohman, M. A. and T. J. Biden
*Journal of Biological Chemistry* 279, 27534-27541.

**Diabetes Obesity Clinical Group**
**Associate Professor Katherine Samaras (k.samaras@garvan.org.au)**
**Ph: (02) 9295 8312**

**Project 1: Adipose tissue biology in obesity**
Obesity is Australia's major health problem impacting on every aspect of health, particularly diabetes and heart disease. Adipokines and inflammatory cytokines from adipose tissue affect tissues locally and metabolism systemically. We are currently examining how obesity affects aspects of metabolism and inflammation. This project focuses on mechanisms by which diet and weight loss affects adipose tissue biology, inflammation, lipid and glucose metabolism and insulin action. The project involves both human and animal experiments.

**Project 2: Glucose and lipid metabolism in treated HIV**
Treatment of HIV infection is associated with metabolic complications including hyperlipidemia and insulin resistance. Clinically, studies have shown these rapidly translate to diabetes and heart disease, with an accelerated pathogenesis. This project investigates how the drugs used to effectively treat HIV-infection promotes diabetes and lipid disturbances, using in vitro and in vivo model

**Molecular Metabolism Group**
**Associate Professor Greg Cooney (g.cooney@garvan.org.au) Ph: (02) 9295 8209**
**Dr Nigel Turner (n.turner@garvan.org.au) Ph: (02) 9295 8208**
**Dr Bronwyn Hegarty (b.hegarty@garvan.org.au) Ph: (02) 9295 8223**

Increased body fat (obesity) is one of the most important current health problems because obesity is associated with the development of a number of serious and common diseases such as heart disease, stroke, type 2 diabetes, liver disease, arthritis and cancer. The broad aim of our projects is to understand how different tissues and different genes contribute to the way the body balances food intake and energy expenditure to maintain healthy body weight and what goes wrong when this balance breaks down and obesity develops.

**Project 1: Circadian Rhythms and energy metabolism**
Many important genes of metabolism are expressed in a circadian rhythm synchronized with the light/dark cycle and feeding/sleeping patterns. The modern lifestyle is associated with disrupted eating and sleeping patterns and an increase in obesity but whether this is accompanied or caused by a disruption in the circadian rhythms of gene expression is not known. This project investigates whether circadian gene expression is altered in situations of obesity and insulin resistance and what metabolic processes these genes regulate in different tissues.

**Project 2: Mitochondrial metabolism and insulin resistance**
Another research project is aimed at determining the role of muscle mitochondrial capacity in regulating glucose and lipid metabolism. In muscle the fibre type and number of mitochondria are correlated with insulin sensitivity. This project examines the importance of mitochondrial dysfunction in the genesis of insulin resistance and investigates whether increasing mitochondrial number by over-expressing mitochondrial transcription factors can also improve insulin action in muscle.

**Project 3: Liver metabolism and energy homeostasis**
The liver integrates signals from nutrients and hormones to play a key role in the maintenance of fuel homeostasis. During fasting, the liver releases glucose into the bloodstream, whereas under fed conditions hepatic glucose output is inhibited and the liver converts excess energy to glycogen and triglycerides. A recently identified hormone 'adiponectin', which is secreted by fat tissue, has been shown to target the liver and have beneficial effects on hepatic metabolism and whole-body insulin action thought to be mediated through its activation of AMP-activated protein kinase (AMPK), a key enzyme which regulates pathways involved in both fat and glucose metabolism. However, we have recently shown that important AMPK-independent effects also exist. This project aims to identify the mechanisms by which adiponectin alters hepatic metabolism and the signalling pathways involved.

**Project 4: Metabolic phenotyping**
We are also investigating how tissue specific deletion of the genes c-Cbl, acetyl CoA carboxylase (ACC) Grb10 and Grb14 in mice contribute to lean and insulin sensitive phenotypes with the aim of identifying novel pathways for regulating energy expenditure, body fat and insulin action.

**Recent publications.**
Kraegen EW, Cooney GJ. Free fatty acids and skeletal muscle insulin resistance. *Curr Opin Lipidol.* 2008 19:235-41.

Turner N, Bruce CR, Beale SM, Hoehn KL, So T, Rolph MS, Cooney GJ. Excess lipid availability increases mitochondrial fatty acid oxidative capacity in muscle: evidence against a role for reduced fatty acid oxidation in lipid-induced insulin resistance in rodents. *Diabetes.* 2007 56(8):2085-92.

Cleasby ME, Davey JR, Reinten TA, Graham MW, Jame DE, \ Kraegen EW and Cooney GJ. Acute bidirectional manipulation of muscle glucose uptake by *in vivo* electro-transfer of constructs targeting glucose transporter genes. *Diabetes* 54:2702-2711, 2005.

Molero, J.C., Jensen, T.E., Withers, P.C., Couzens, M., Herzog, H., Thien, C.B., Langdon, W.Y., Walder, K., Murphy, M.A., Bowtell, D.D., James, D.E., Cooney, G.J. c-Cbl-deficient mice have reduced adiposity, higher energy expenditure, and improved peripheral insulin action. *J Clin Invest*. 114:1326-1333, 2004.

**Cooper Group**
**Dr Antony Cooper (a.cooper@garvan.org.au)**
**Ph: (02) 9295 2838**

**Project 1: Elucidating the underlying molecular mechanism for the neurodegenerative disease ALS / Motor Neuron Disease.**

ALS is a devastating neurodegenerative disorder that attacks motor neurons leading to paralysis and death within 2-5 years after the onset of the disease and for which there are no effective therapies. ALS pathological and clinical features including abnormal proteinaceous accumulations (aggregates/inclusions) of misfolded mutant superoxide dismutase (SOD1) that mark degenerating motor neurons. No effective treatment exists for ALS so determining the underlying cellular molecular mechanism(s) is critical as a basis for developing a treatment or cure.

To identify the pathological mechanism of ALS we have developed a model system expressing lethal levels of mutant aggregating SOD1. We are currently screening 5000 genes for those whose overexpression suppress or enhance SOD1 toxicity. The identification of these genes will indicate both the dysfunctional molecular process responsible *and* identify genes whose modulation of expression may have therapeutic value. The project will first involve bioinformatic analysis to identify common pathways amongst the genes identified and then confirming the role of these pathways in our model system before expanding the analysis into cell culture, mouse and human models of ALS.

Our discoveries will have far reaching outcomes in terms of identifying the underlying disease molecular mechanisms and indicate avenues best suited for therapeutic intervention.

Project 2: **Identification of Genetic Risk Factors for Parkinson's Disease (PD)**

PD is a neurodegenerative disease affecting >50,000 Australians who have already lost ~ 40% of dopamine producing neurons at time of diagnosis. Earlier diagnoses and new treatments are needed as current therapies are only partially effective.

αSynuclein is a natively non-toxic protein of unknown function that associates with synaptic vesicles. αSynuclein is also the central component in PD as its aggregated form is the main component of Lewy bodies, the primary pathological hallmarks of PD. Although the underlying cause of PD is unknown in >90% of cases the propensity of αSynuclein to become cytotoxic likely results from a complex interaction of unknown predisposing genes (risk factors). Identifying inherited risk factors will enhance our understanding of how Parkinson's disease develops and is an important step towards preventing the disease or to develop therapeutic agents that may inhibit the degeneration of neurons.

Using a new model system expressing nontoxic levels of αSynuclein, we screened for αSynuclein dependent toxicity upon loss of function of 5000 genes. We have identified the critical role of mitochondrial dysfunction as well as major signalling pathways. This project will investigate a group of these genes to identify the underlying molecular mechanism and then test in human brain samples.