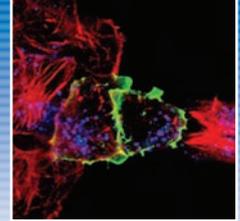


# Diabetes and Obesity Research Program



## Molecular Metabolism Group

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### BACKGROUND

Increased body fat (obesity) is one of the most important 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, arthritis, and cancer. The broad aim of our projects is to understand how 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 synchronised 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 whether changing the expression of circadian genes can alter susceptibility to obesity.

### PROJECT 2 Stress response genes and insulin resistance

There is considerable evidence that obesity is associated with an increased inflammatory response and insulin resistance but the extent to which inflammatory responses cause insulin resistance is not clear. To determine the role of specific inflammatory genes in insulin resistance this project will use electroporation gene transfer to overexpress or reduce expression of specific genes both *in vitro* and *in vivo* and examine the effect on glucose and lipid metabolism.

### PROJECT 3 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 will determine whether increasing mitochondrial number by over-expressing mitochondrial transcription factors can also improve insulin action in muscle.

### PROJECT 4 Metabolic phenotyping

We are also investigating how deletion of the genes c-Cbl and Grb14 in mice contribute to lean and insulin sensitive phenotypes (respectively) with the aim of identifying novel pathways for regulating energy expenditure, body fat and insulin action.

### Molecular Metabolism Selected Publications

Molero JC, Jensen TE, Withers PC, Couzens M, Herzog H, Thien CB, Langdon WY, Walder K, Murphy MA, Bowtell DD, James DE, Cooney GJ. c-Cbl-deficient mice have reduced adiposity, higher energy expenditure, and improved peripheral insulin action. *J Clin Invest* 2004; 114:1326-1333.

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

Parkes HA, Preston E, Wilks D, Ballesteros M, Carpenter L, Wood L, Kraegen EW, Furler S, Cooney GJ. Over-expression of Acyl-CoA Synthetase-1 increases lipid deposition in hepatic (HepG2) cells and rodent liver *in vivo*. *Am J Physiol Endocrinol Metab* 2006 May 16; [Epub ahead of print].



## Phospholipid Biology Group

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### BACKGROUND

It is critical for the function of multicellular organisms that signals triggering intracellular events are transmitted across biological membranes. It is becoming increasingly clear, however, that the biological membrane is not just a passive barrier across which a signal must pass. The phospholipid components of biological membranes play varied key roles in transducing, attenuating and appropriately locating the signalling events within a cell.

Phospholipid membranes also define compartments and organelles within cells. What is now also becoming clear is that the phospholipid composition of these membranes is dynamic and regulated changes

allow communication and trafficking between intracellular compartments, changes that are critical for cellular processes such as secretion or exocytosis.

We are interested in the role that phospholipids, and the enzymes responsible for their production and destruction, have to play in both the signalling and physical control of intracellular trafficking events; in particular, the events controlling insulin exocytosis from pancreatic beta-cells. Phospholipids participate in the signal transduction cascade triggering insulin release and in the fusion event between insulin vesicles and the plasma membrane. Also under investigation are the roles phospholipids play in controlling insulin-stimulated signalling cascades and trafficking events in muscle cells and adipocytes.

We use cell biological (particularly live cell microscopy) and biochemical approaches to identify where, when and why specific phospholipids are produced or destroyed, how they participate in signalling events and regulate intracellular trafficking. We are interested to discover how these processes may be disrupted in disease, particularly diabetes.

## PROJECT

1

### The role of phospholipase D (PLD) in signal transduction and membrane fusion

PLD hydrolyses a major membrane phospholipid phosphatidylcholine to produce the highly 'bioactive' signalling phosphatidic acid. We believe phosphatidic acid can act as a signalling molecule and also directly affect membrane fusion, and want to investigate this hypothesis.

## PROJECT

2

### The role of Phosphatidylinositol 3-kinases (PI3-K) in insulin signal transduction.

This project will look at the process whereby PI3-Ks phosphorylate membrane phospholipids generate potent signalling molecules which we think are responsible for various controls on intracellular vesicle trafficking.

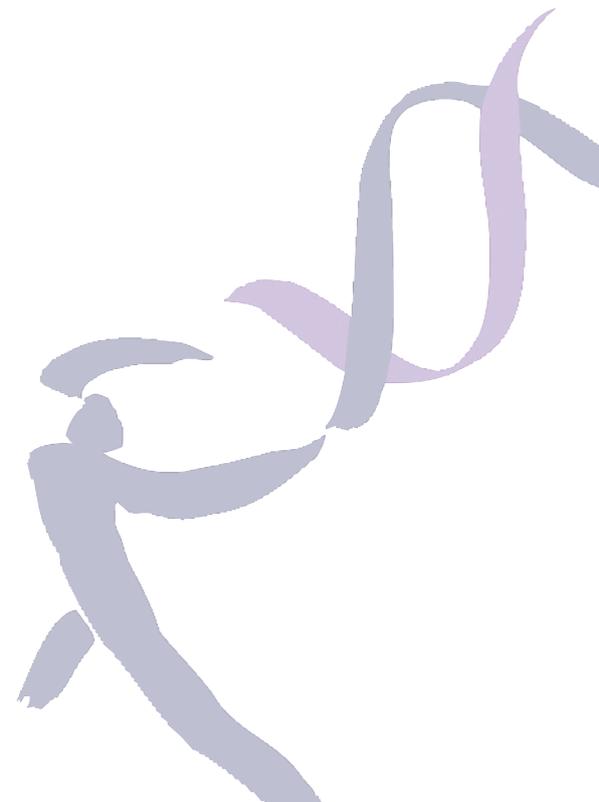
### Phospholipid Biology Group Selected Publications

Cazzolli R, Shemon AN, Fang MQ, Hughes WE. Phospholipid signalling through phospholipase D and phosphatidic acid. *IUBMB Life* 2006; (In press).

Cazzolli R, Huang P, Teng S, Hughes WE. Measuring phospholipase D activity in insulin secreting pancreatic beta-cells and insulin responsive muscle cells and adipocytes. *Meth Molec Biol* 2006; (In press).

Hughes WE, Elgundi Z, Huang P, Frohman MA, Biden TJ. Phospholipase D1 regulates secretagogue-stimulated insulin release in pancreatic beta-cells. *J Biol Chem* 2004; 279:27534-41.

Hughes WE, Larijani B, Parker PJ. Detecting protein-phospholipid interactions: EGF-induced activation of Phospholipase D1b in situ. *J Biol Chem* 2002; 277, 22974-22979.





**DIABETES SIGNALLING UNIT**  
**Insulin Signalling Group**

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**BACKGROUND**

Type 2 diabetes is a major disease with debilitating complications, and its prevalence is reaching epidemic proportions. This group focuses on diabetes as a function of signal transduction pathways, and our work spans the key tissues involved in glucose homeostasis. Diabetes is strongly associated with obesity, and our strengths in signalling and lipid biochemistry enable us to study the molecular mechanisms of lipid-induced insulin resistance in the target tissues of the hormone, such as the identification of key metabolites that accumulate in skeletal muscle, and how these disrupt insulin receptor signalling cascades. We are also investigating the regulation of insulin secretion from the pancreatic beta-cell, specifically the roles of lipid metabolites in causing alterations in gene transcription and the activation of stress pathways which trigger beta-cell apoptosis and de-differentiation. Emphasis is on the role of the lipid-activated protein kinase C (PKC) family of signalling enzymes in the dysregulation of whole body glucose homeostasis and elucidation of the diverse mechanisms by which it exerts its effects.

**PROJECT 1** **PKC epsilon signal transduction in beta-cells and liver**

Using knockout mice, we have discovered new roles for PKC epsilon, in beta-cell dysfunction and in the clearance of insulin by the liver. Our current aims are to understand the underlying intracellular mechanisms, using our expertise in gene chip technologies, gene over-expression or knock down, and protein phosphorylation and proteomics. We wish to identify the signalling molecules, protein substrates and binding partners which act in concert with PKC epsilon in order to define new pathways in beta-cells and liver. Tissue-specific PKC epsilon knockout mice will be available to confirm the importance of these pathways *in vivo*.

**PROJECT 2** **The role of PKC epsilon in insulin receptor endocytosis**

Our studies in PKC epsilon knockout mice indicate that this kinase promotes insulin clearance by the liver. Clearance by hepatocytes involves internalisation of insulin, bound to its receptor, and subsequent degradation. PKC epsilon has been implicated in cytoskeletal rearrangement and in receptor-mediated

endocytosis in other systems, and we will now examine its role in the trafficking of the insulin receptor. We have established immortalised cell lines as well as primary hepatocytes from wild type and knockout mice, which will be used to set up models and assays to address the interaction between PKC epsilon and endocytotic machinery. This is a joint project with the A/Prof Trevor Biden's Beta Cell Signalling Group.

**PROJECT 3** **Lipid-induced insulin resistance in skeletal muscle**

Our work using cultured skeletal muscle cells treated with fatty acids as a model of obesity and insulin resistance has identified novel lipid intermediates which inhibit insulin signal transduction, including ceramide and phosphatidic acid. However, the mechanisms by which these species interfere with normal insulin action are not clear. Further work will investigate the activation of inhibitory pathways and gene expression by such lipids which in turn act to block insulin signalling and normal glucose metabolism.

**Diabetes Signalling Unit Selected Publications**

Busch AK, Cordery D, Denyer GS, Biden TJ. Expression profiling of palmitate- and oleate-regulated genes provides novel insights into the effects of chronic lipid exposure on pancreatic b-cell function. *Diabetes* 2002; 51:977-987

Schmitz-Peiffer C, Craig DL, Biden TJ. Ceramide generation is sufficient to account for the inhibition of the insulin-stimulated PKB pathway in C2C12 skeletal muscle cells pretreated with palmitate. *J Biol Chem* 1999; 274:24202-24210

Cazzolli R, Carpenter L, Biden TJ, Schmitz-Peiffer C: A role for protein phosphatase 2A-like activity, but not atypical protein kinase C zeta, in the inhibition of protein kinase B/Akt and glycogen synthesis by palmitate. *Diabetes* 2001; 50:2210-18

Burchfield JG, Lennard AJ, Narasimhan S, Hughes WE, Wasinger VC, Corthals GL, Okuda T, Kondoh H, Biden TJ, Schmitz-Peiffer C: Akt mediates insulin-stimulated phosphorylation of Ndr2- Evidence for cross-talk with protein kinase C theta. *J Biol Chem* 2004; 279:18623-32

