long-term consequences. In rodents, manipulating the microbial community structures that constitute the intestinal microbiota can impact body composition, but how this information may translate to humans is still unclear. The realisation that brown-like adipose tissue exists in humans has prompted provocative studies in animals demonstrating that adipose depots can be induced to carry out inefficient metabolism, a process that if translated to humans could alter energy balance to treat obesity and diabetes. A common obesity complication is type 2 diabetes, but obesity does not universally lead to diabetes, providing some support for the notion of ''healthy'' obesity. For those with obesity-associated diabetes, recent therapeutic options appear to decrease certain diabetes complications although the responsible mechanisms are poorly understood. Emerging evidence suggests that a combination of genetic and metabolic profiling could help guide management, but such an approach will also require behavioural and population-based strategies to address the failure of many providers and patients to utilise proven therapies.

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Increased intestinal permeability as a risk factor for type 2 diabetes in obesity

Amanda J. Cox[2,1,*], Ping Zhang[2,1], Donald W. Bowden[3], Benedict Devereaux[4], Peter M. Davoren[5], Allan W. Cripps[2,1], Nicholas P. West[2,1]

[1] *Menzies Health Institute Queensland, Southport, QLD, Australia*
[2] *Griffith University, Southport, QLD, Australia*
[3] *Centre for Diabetes Research, Wake Forest School of Medicine, Winston-Salem, NC, USA*
[4] *Digestive Diseases QLD, Chermside, QLD, Australia*
[5] *Diabetes and Endocrinology, Gold Coast University Hospital, Southport, QLD, Australia*

Introduction: The interplay between the gut microbiota, intestinal permeability and chronic low grade inflammatory responses in the context of risk for obesity-associated disease continue to be of interest. A permeable intestinal mucosa is necessary to facilitate critical absorptive functions, but alterations in intestinal permeability have the potential to trigger Metabolic Endotoxaemia (ME) which may result in downstream activation of inflammatory signalling pathways and contribute to risk for disease. The aim of the study was to examine the associations between intestinal permeability and type 2 diabetes (T2D) using a derived risk score approach.

Methods: A total of 130 individuals with T2D (age: 57.5 ± 6.2 years (mean ± SD); BMI: 30.4 ± 3.2; 45% female) and 161 individuals without T2D (age: 37.4 ± 12.5 years; BMI: 25.1 ± 3.9; 65% female) were included in the study. Assessment of intestinal permeability included measurement of circulating lipopolysaccharide (LPS), LPS-binding protein (LBP) and intestinal fatty acid binding protein (iFABP) concentrations which were then used for calculation of a derived permeability risk score (PRS) based on quartile scoring of each individual measure. Associations between the PRS and T2D status were assessed using logistic regression models.

Results: LBP (~34%, $p < 0.001$), iFABP (~46%, $p < 0.001$) and the PRS (~24%, $p < 0.001$) were all significantly higher in the T2D affected individuals. Quantification of risk across PRS tertiles revealed that individuals with a PRS in the upper tertile were 5.07 times more likely (CI: 1.72—14.95; $p = 0.003$) to have T2D independent of age, sex and BMI.

Conclusions: These data support an association between intestinal permeability and risk for T2D. Consideration of intestinal permeability assessment as a potential tool for classifying individuals with Metabolic Syndrome as high or low risk for T2D development appears a logical progression of this work.

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Insulin transport and activity in the central nervous system

Denovan P. Begg

University of New South Wales, UNSW, Sydney, NSW, Australia

Insulin acts within the central nervous system to alter numerous physiological outcomes including energy balance and glucose homeostasis. Insulin is transported into the central nervous system by a saturable-receptor mediated process that is proposed to be dependent on the insulin receptor. Transport of insulin into the brain is altered by numerous factors including diet induced obesity [1]. It has previously been observed that the

weight sparing effect of detemir insulin, relative to other long-acting insulin formulations, is associated with increased transport into the central nervous system [2]. We hypothesised that the effects of detemir insulin on energy balance would be mediated by an increase in central nervous system insulin signalling. Chronic treatment with detemir insulin resulted in reductions in both food intake and weight gain relative to insulin glargine or normal insulin treatment in C57BL/6J mice. Acute peripheral detemir insulin treatment resulted in reduced food intake, with increased phosphorylated Akt also observed in the arcuate nucleus of the hypothalamus of detemir insulin treated mice, relative to other insulin treatments. When mice were maintained on a high fat diet the acute effects of detemir insulin on both energy balance and phosphorylated Akt were inhibited. Furthermore, when specific neuronal populations of insulin receptors were knocked out, animals were insensitive to the acute effects of detemir insulin on energy balance. These data demonstrate that detemir insulin reduces weight gain by acting on the central nervous system to reduce food intake. The inhibition of this effect in high fat diet treated animals indicates that detemir insulin is subject to resistance of insulin transport into the brain.

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Signal transduction pathways activated by the orexigenic gut derived hormone insulin-like peptide 5 at relaxin family peptide receptor 4

Dana S. Hutchinson*, Sheng Y. Ang, Bronwyn A. Evans, Roger J. Summers, Martina Kocan

Monash University, Parkville, VIC, Australia

Introduction: Insulin-like peptide 5 (INSL5) is a two-chain, three-disulphide bonded peptide belonging to the insulin/relaxin superfamily expressed in the enteroendocrine L-cells of human and mouse colon [1]. It is the cognate ligand for relaxin family peptide receptor 4 (RXFP4) a GPCR mainly expressed in the colorectum and enteric nervous system. Currently little is known of the signal transduction pathways activated by RXFP4.

Aims: This study examined intracellular signalling pathways activated by INSL5 acting at the human RXFP4 receptor stably expressed in CHO cells.

Methods: Cell signalling was investigated using AlphaScreen® assays. Ca^{2+} flux was monitored in a Flexstation® using X-rhod-1AM. RXFP4 recruitment of $G\alpha_{i/o}$ protein isoforms were determined by rescue of ERK1/2 responses by PTX-insensitive $G\alpha_{i/o}$ C351I mutants ($mG\alpha_{i/o}$). Cell proliferation was studied by bromo-deoxyuridine (BrdU) cell proliferation ELISA. RXFP4 interactions with β-arrestins 1/2, G protein-coupled receptor kinase 2 (GRK2), KRas and Rab5a were examined using real-time BRET.

Results: Mouse INSL5 inhibited forskolin-stimulated cAMP accumulation and activated ERK1/2, p38MAPK, Akt-Ser473, Akt-Thr308 and S6 ribosomal protein (S6RP) more potently than human INSL5. No Ca^{2+} mobilisation was observed. PTX-abolished INSL5-stimulated ERK1/2 signal was rescued by $mG\alpha_{oA}$, $mG\alpha_{oB}$, $mG\alpha_{i2}$ and to a lesser extent by $mG\alpha_{i1}$ and $mG\alpha_{i3}$. RXFP4 interacted with GRK2, β-arrestins 1/2 and Rab5a but dissociated from KRas.

Discussion: INSL5 negatively regulates cAMP production and activates multiple signalling pathways important for diverse cellular functions including growth, differentiation and proliferation (ERK1/2, p38MAPK, Akt) and protein synthesis (S6RP). Following INSL5 activation, RXFP4 recruits a variety of $G\alpha_{i/o}$ and is regulated by β-arrestin 1/2 and GRK2 leading to receptor internalisation. Information on