75

Identification and characterisation of metabolically distinct human adipocyte precursor cells

Arthe Raajendiran[1,2,*], Geraldine Ooi[3,4], Ashlee Clark[2], Jacqueline Bayliss[1,2], Paul O'Brien[4], Renea Taylor[2], Matthew Rodeheffer[5], Paul Burton[3,4], Matthew Watt[1,2]

[1] *Physiology, The University of Melbourne, Parkville, VIC, Australia*
[2] *Monash Biomedicine Discovery Institute, Monash University, Clayton, VIC, Australia*
[3] *Upper Gastro-Intestinal Surgery, The Alfred, Melbourne, VIC, Australia*
[4] *Centre for Obesity Research & Education, Monash University, Melbourne, VIC, Australia*
[5] *Department of Molecular Cell and Developmental Biology, Yale University, New Haven, CT, USA*

An enlarged adipose tissue (AT) mass, as seen in obesity, is related to the onset of insulin resistance that further perpetuates a constellation of life-threatening metabolic diseases such as type 2 diabetes, cardiovascular diseases and some forms of cancer. While understanding the expansion of a regional adipose tissue mass helps to delineate the specific cellular processes promoting health and disease, the adipocytes regulating the mass of an AT depot are differentiated from the tissue-resident adipocyte precursor cells (APCs) whose identity in humans remain largely unknown. Hence, we aimed to identify the human APCs located within the regional AT depots obtained from visceral, abdominal subcutaneous and gluteal-femoral regions to assess their adipogenic potential, metabolic properties and any correlations with metabolic disease.

We have discovered that human AT consists of three transcriptionally (RNASeq) distinct APCs in the lineage depleted ($CD31^{-}CD45^{-}$) stromal vascular fraction (SVF) using fluorescent activated cell sorting technology and termed them as $CD34^{-}$, $CD34^{lo}$ and $CD34^{hi}$ APCs. All three precursor cells differentiated into mature adipocytes *in vitro*. Freshly sorted human $CD34^{hi}$ APCs formed mature adipocytes when xenotransplanted into immunodeficient mice and the assessment of *in vivo* adipogenesis of $CD34^{-}$ and $CD34^{lo}$ APCs is still ongoing.

Rates of lipolysis, fatty acid uptake were higher in $CD34^{hi}$ compared with $CD34^{-}$ and $CD34^{lo}$ APC-derived adipocytes *in vitro*, the latter having very low fatty acid turnover. Interestingly, only the $CD34^{-}$ adipocytes displayed enhanced thermogenic potential *in vitro*. Importantly, the proportion of $CD34^{hi}$ APCs was higher and $CD34^{-}$ APCs was lower in the AT of individuals with type 2 diabetes, suggesting that dysregulated lipolysis commonly observed in these individuals may be attributed to alterations in APC abundance.

Summarily, we have identified three distinct *bona fide* APCs varying in their metabolic capacities and distribution in metabolically healthy and non-healthy adipose tissue depots.

Funding: NHMRC, Australia.

https://doi.org/10.1016/j.orcp.2018.11.080

76

Targeting mitochondrial uncoupling to reverse obesity and related comorbidities

Stephanie J. Alexopoulos[1], Sing-Young Chen[1], Elizabeth S. Childress[2], Joseph Salamoun[2], Divya P. Shah[1], Webster L. Santos[2], Kyle L. Hoehn[1,*]

[1] *University of New South Wales, Kensington, NSW, Australia*
[2] *Virginia Tech, Blacksburg, VA, United States*

Small molecule mitochondrial uncouplers decrease oxidative efficiency resulting in increased nutrient oxidation and decreased mitochondrial reactive species production. Mitochondrial uncouplers have demonstrated anti-obesity effects in humans and protect from fatty liver disease in rodents. However, most mitochondrial uncouplers have limited use in the clinic due to their narrow therapeutic window between efficacy and toxicity. The on-target activity of mitochondrial uncouplers is the mitochondrial inner membrane; however, most uncouplers have unwanted off-target activity at other cellular membranes resulting in undesired effects including plasma membrane depolarization. Recently, we identified several new classes of mitochondrial uncouplers that do not depolarize the plasma membrane at concentrations needed to drive maximal mitochondrial respiration. Several molecules reverse obesity and insulin resistance in mice fed a Western Diet, and decrease non-alcoholic fatty liver disease activity score by 2 points in a mouse model of steatohepatitis. Importantly, these effects occur without decreased food intake or loss of lean body mass. These new mitochondrial uncouplers have a broad therapeutic window *in vivo* and may represent new therapeutic treatments for obesity and its related metabolic disorders.

KLH and WLS are co-founders of Continuum Biosciences and declare a commercial interest in the research.

https://doi.org/10.1016/j.orcp.2018.11.081

77

Tissue-specific actions of protein kinase C epsilon in the modulation of insulin signalling and glucose tolerance

Carsten Schmitz-Peiffer

Diabetes and Metabolism Division, Garvan Institute of Medical Research, Sydney, NSW, Australia

Activation of protein kinase C epsilon (PKCε) in the liver has been widely associated with hepatic insulin resistance. PKCε is proposed to inhibit insulin signalling through phosphorylation of the insulin receptor. We tested this directly using "floxed" PKCε mice bred with mice expressing Cre recombinase under the control of the cytomegalovirus, albumin, lysozyme M or adiponectin promoters to generate global, liver-, macrophage- and adipose tissue-specific PKCε knockout (KO) mice. Global deletion of PKCε recapitulated the benefits for diet-induced glucose intolerance that we previously described using conventional PKCε KO mice. However, we did not detect PKCε-dependent alterations in hepatic insulin receptor phosphorylation. Neither macrophage- nor liver-specific KO mice were protected against diet-induced glucose intolerance or insulin resistance determined by euglycemic clamp. In contrast, adipose tissue-specific KO mice did exhibit improved glucose tolerance, but phosphoproteomics revealed no PKCε-dependent effect on the activation of canonical insulin signaling pathways. Instead, we observed altered phosphorylation of proteins associated with cell

junctions and endosomes, suggesting a role in endocrine function. This was linked to changes in hepatic expression of several genes linked to glucose homeostasis and lipid metabolism. The primary effect of PKCε on glucose homeostasis is, therefore, not exerted directly in the liver as currently posited. However, PKCε activity in adipose tissue modulates glucose tolerance and is involved in crosstalk with the liver.

https://doi.org/10.1016/j.orcp.2018.11.082

78

Lipidomic profiling reveals early-stage metabolic dysfunction in overweight or obese humans

Aya Mousa[1,*], Negar Naderpoor[1], Natalie Mellett[2], Kirsty Wilson[3], Peter J. Meikle[4], Barbora De Courten[1], Magdalena Plebanski[3,5]

[1] *Monash Centre for Health Research and Implementation, School of Public Health and Preventive Medicine, School of Public Health and Preventive Medicine, Melbourne, VIC, Australia*
[2] *Metabolomics Laboratory, Baker Heart and Diabetes Institute, Melbourne, VIC, Australia*
[3] *Department of Immunology and Pathology, Monash University, Melbourne, VIC, Australia*
[4] *Metabolomics Laboratory, Baker Heart and Diabetes Institute, Melbourne, VIC, Australia*
[5] *School of Health and Biomedical Science, RMIT University, Melbourne, VIC, Australia*

Advances in mass spectrometry and lipidomics techniques are providing new insights into the role of lipid metabolism in obesity and its metabolic sequelae. However, human lipidomic studies have produced inconsistent results, owing in part to the use of indirect proxy measures of obesity and insulin resistance and the relatively limited coverage of the lipidome. Here, we explored the relationship between the plasma lipidome and metabolic profiles using direct gold-standard measures of adiposity, insulin sensitivity, and insulin secretion, in addition to comprehensive lipidomic profiling (>450 species) and measurement of inflammatory cytokines and adipokines. We present new evidence showing a strong and independent positive correlation between the lysophosphatidylinositol (LPI) lipid class and insulin secretion *in vivo* in humans, supporting the insulinotropic effects of LPI demonstrated in mouse islets. Dihydroceramide, a sphingolipid precursor, was independently and negatively correlated with insulin sensitivity, indicating a possible upregulation in sphingolipid synthesis in obese individuals. We also show that phosphatidylethanolamine and its vinyl ether-linked (plasmalogen) derivatives correlate negatively with body fat, while dihexosylceramide correlates positively with interleukin-10. Together, these lipid classes may signify early pathogenesis toward type 2 diabetes and could serve as novel therapeutic targets or biomarkers for identification of high-risk individuals.

https://doi.org/10.1016/j.orcp.2018.11.083

79

Insights into type 2 diabetes susceptibility and resilience: TOFI_Asia study

Ivana R. Sequeira[1,*], Wilson Yip[1], Louise W.W. Lu[1], Lindsay Plank[2], Rinki Murphy[3], Kieren G. Hollingsworth[4], Jun Lu[5], Sally D. Poppitt[1,3]

[1] *Human Nutrition Unit, School of Biological Sciences, University of Auckland, Auckland, New Zealand*
[2] *Body Composition Unit, Department of Surgery, University of Auckland, Auckland, New Zealand*
[3] *Department of Medicine, University of Auckland, Auckland, New Zealand*
[4] *Newcastle Magnetic Resonance Centre, Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, United Kingdom*
[5] *Faculty of Health and Environmental Sciences, AUT, Auckland, New Zealand*

Predicting rate of progression and identifying those most at risk of adverse metabolic health is difficult and often associated with environmental, lifestyle, and dietary factors. Importantly site of lipid deposition, including ectopic 'overspill' into key organs, increases metabolic risk. We investigated susceptibility to type 2 diabetes (T2D) in healthy and prediabetic Asian Chinese ($N = 209$) and European Caucasian ($N = 156$) adults resident in Auckland, enrolled in the TOFI_Asia Study; within the Peak Nutrition for Metabolic Health (PANaMAH) program, one of four priority research platforms in the New Zealand National Science Challenges (NSC).

Phenotypic characterisation of the 2 ethnicity cohorts, using anthropometry, body composition (dual energy X-ray absorptiometry, DXA) and blood biochemistry revealed that although of similar age and body mass index (BMI), Chinese (Mean ± SEM, 42 ± 1 yrs; 27.4 ± 0.3 kg/m^2) had significantly greater abdominal fat (42.0 ± 0.6% vs 37.0 ± 1.1%, $p < 0.01$) than Caucasian (43 ± 1 yrs; 26.9 ± 0.4 kg/m^2) with correspondingly higher fasting plasma glucose (FPG) concentrations (5.4 ± 0.04 mmol/l vs 5.0 ± 0.04 mmol/l, $p < 0.001$) and glycated haemoglobin, Hb_{A1c} (36.0 ± 0.3 mmol/mol vs 33.0 ± 0.3 mmol/mol, $p < 0.05$). In addition, pancreatic and liver fat were quantified, using magnetic resonance imaging (MRI) and spectroscopy (MRS), in a subset of 36 Chinese (41 ± 2 yrs; 26.9 ± 0.7 kg/m^2) and 34 Caucasian (48 ± 3 yrs; 28.0 ± 0.7 kg/m^2) women from the cohort. These overweight, younger Asian Chinese had significantly lower MRI-determined abdominal and subcutaneous fat ($p < 0.05$) but pancreas (4.3 ± 0.3%) and liver (11.0 ± 1.7%) fat matched that, or tended to be higher than, Caucasian (pancreas: 4.1 ± 0.3%; liver: 8.1 ± 1.7%). Importantly, fat in pancreas (Chinese: $R^2 = 0.10$, $p = 0.09$; Caucasian: $R^2 = 0.28$, $p = 0.001$) and liver (Chinese: $R^2 = 0.12$, $p = 0.04$; Caucasian: $R^2 = 0.11$, $p = 0.05$) were positively correlated with FPG. The **T**hin on the **O**utside **F**at on the **I**nside 'TOFI' profile observed in this Asian Chinese cohort, may contribute to their greater risk of poor metabolic health compared to Caucasian counterparts even in individuals of the same BMI and younger age.

https://doi.org/10.1016/j.orcp.2018.11.084