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Identification and characterisation of metabolically distinct human adipocyte precursor cells

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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.

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Targeting mitochondrial uncoupling to reverse obesity and related comorbidities

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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.

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

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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