

246d Adipocyte Metabolic Engineering for Increased Fatty Acid Oxidation through Uncoupling Protein over-Expression

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Obesity is a chronic condition that primarily develops from an increase in body fat in the form of white adipose tissue (WAT) mass. The resulting adiposity is a risk factor for many diseases, including type 2 diabetes (T2D), cardiovascular diseases, and some forms of cancer. In particular, obesity-induced insulin resistance is a major contributor to T2D pathogenesis. In this regard, reducing WAT mass by targeted modulation of metabolic enzymes in fat cell metabolism is an attractive molecular therapeutic alternative to dietary approaches. Expansion of WAT mass involves both increases in fat cell size (hypertrophy) and cell number (hyperplasia). The latter occurs through recruitment and differentiation of precursor cells (adipogenesis). Findings from an earlier metabolic profiling study conducted in our laboratory suggested that the intracellular free fatty acid content is a key marker for adipogenesis as well as subsequent enlargement of fat cell size through triglyceride (TG) accumulation. In the present study, we exogenously up-regulate a novel respiratory uncoupling protein to increase substrate oxidation, and thereby control adipocyte fatty acid content.

Increasing molecular evidence points to a family of uncoupling proteins (UCPs) playing an important role in adipocyte fat metabolism. Of specific interest is UCP1, which in brown adipocytes mediates energy dissipation as heat by de-coupling respiration and ATP synthesis. UCP1 is minimally expressed in white adipose tissue (WAT). We hypothesize that controlled expression of UCP1 in WAT will result in enhanced fatty acid oxidation to compensate for reduced ATP synthesis. We used a Tet-Off retroviral transfection system to express UCP1, with doxycycline being used to control the extent of expression. UCP1 cDNA was cloned into pRevTRE and was stably transfected into 3T3-L1 preadipocytes prior to differentiating them into adipocytes. A reporter gene (EGFP) was also transfected in parallel to optimize the transfection and preadipocyte differentiation conditions as well as to demonstrate regulated expression. Morphological analyses, Oil-Red-O, and biochemical assays on adipocyte differentiation markers indicated that the gene transfection procedure did not influence normal differentiation. Metabolite measurements showed that the UCP1-expressing adipocytes accumulated 83% less triglyceride and 85 % free fatty acids while maintaining constant ATP levels. These results suggest UCP1 and other metabolic enzymes as potential targets for development of pharmacological agents for the treatment of obesity and related disorders.