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Succination Impairs Protein Folding and Promotes CHOP Stability in the Adipocyte During Diabetes.

Type 2 diabetes mellitus has been diagnosed in ~21 million people in the United States and is closely correlated with obesity, prompting the need for a detailed understanding of adipocyte metabolism in the development of diabetes. The intake of excess nutrients surpasses the energy requirements of the cell and leads to increased mitochondrial stress in the adipocyte. We have shown that this is associated with increased levels of the mitochondrial metabolite fumarate. Fumarate can react with cysteine thiol groups to form the chemical modification S-(2-succino)cysteine (2SC), also termed protein succination. Succination is significantly increased in the adipose tissue of type 2 diabetic mouse models (*ob/ob* and *db/db*) and in adipocytes matured in high glucose medium, resulting in impaired protein function. The endoplasmic reticulum (ER) oxidoreductase protein disulfide isomerase (PDI) is succinated in adipocytes matured in high glucose, and we investigated if succination would alter PDI oxidoreductase activity, directly linking mitochondrial stress and ER stress. PDI is succinated by fumarate on both CXXC containing active sites, contributing to reduced enzymatic activity. In the presence of prolonged ER stress the unfolded protein response (UPR) triggers the production of the pro-apoptotic protein C/EBP homologous protein (CHOP). Succinated PDI has decreased reductase activity in adipocytes matured in high glucose, and in *db/db* epididymal adipose tissue, in association with increased levels of the ER stress marker CHOP. PDI succination and ER stress were decreased, and PDI reductase activity was restored when exposure to chronic high

glucose was limited, highlighting the importance of calorie restriction in the improvement of adipocyte metabolic function. The experiments completed in Chapter 2 confirm succination of PDI as a novel biochemical mechanism linking altered mitochondrial metabolism to perturbed ER proteostasis in the adipocyte during diabetes.

Our observations in Chapter 2 consistently demonstrated that CHOP levels are elevated in all cases where fumarate and protein succination are increased. Here we show that CHOP levels are significantly increased in adipocytes matured in high glucose in the absence of UPR signaling and with no sign of apoptosis. We propose that the post-translational modification 2SC may be an alternative physiological regulator of CHOP stability under diabetic conditions. Kelch-like ECH-associated protein 1 (Keap1) negatively regulates CHOP degradation in the adipocyte, and Keap1 is succinated in cancers where fumarate levels are elevated. We discovered that while Keap1 is directly succinated in the presence of excess fumarate derived from genetic knockdown methods, it is the oxidative modification of Keap1 that predominates in adipocytes matured in high glucose. Notably, we also determined that succination indirectly regulates CHOP stability through the induction of oxidative stress. The results shown in Chapter 3 demonstrate that increased fumarate induces increased oxidative stress and that the oxidation of Keap1 contributes to sustained CHOP stability and adipocyte dysfunction during diabetes. The studies conducted in Chapters 2 and 3 demonstrate that early biochemical changes in mitochondrial metabolism have important implications for the development of sustained adipocyte stress.