

## TARGETED PROTEOMICS

Can we really live to 150? As recently highlighted by Barbara Walters, caloric restriction and treatment with Resveratrol can impact longevity. These effects have been linked to increased activity of sirtuins, which act to deacetylate nuclear transcription factor proteins and may deacetylate other proteins as well. Loss of protein acetylation leads to increased fatty acid oxidation and gluconeogenesis, however the underlying molecular mechanisms are not well understood. In contrast to caloric restriction, we postulate that sirtuin levels are decreased in obesity, resulting in increased expression of acetylated proteins or protein hyper-acetylation, and surmise that retention of this modification is an important factor in obesity and progression toward associated diseases that ultimately reduce lifespan. In this CNRU Pilot Project work, we observed lower levels of SIRT1, the best characterized of mammalian sirtuins, in the livers of obese mice when compared with lean mice. We then employed targeted proteomics to identify 30 proteins that appeared to be differentially acetylated in response to obesity. Data suggest that the following proteins are hyperacetylated in obesity: 1. Adenosyl homocysteinase is an  $\text{NAD}^+$  dependent enzyme that generates homocysteine from methionine during amino acid metabolism. High levels of homocysteine in blood are a powerful risk factor for cardiovascular disease, progressively and permanently degrading proteins such as collagen, elastin and fibrillin that are important in artery architecture. Even mild hyperhomocysteinaemia induces cardiovascular damage. 2. S-adenosylmethionine synthetase is key in the generation of AdoMet, and the synthesis and breakdown of AdoMet is implicated in the methylation of phospholipids, proteins, DNA, RNA and small molecules. Clearly, disruption in the balance of this process may have a myriad of downstream consequences. 3. Alcohol dehydrogenase 1 and 4. fructose biphosphate aldolase are implicated in gluconeogenesis and glycolysis pathways, respectively. Dysregulation of these pathways may lead to increased hepatic glucose production, a co-morbidity of obesity. 5. Carbamoyl phosphate synthetase catalyzes the first step of the urea cycle. The active sites of this enzyme are connected by a molecular tunnel containing several lysines. It is possible that acetylation of one or more of these lysines influences the metabolism of ammonia through the enzyme, resulting in toxic buildup in the liver. These data help support the hypothesis that increased protein acetylation may trigger progression from obesity to associated disease states. *Results of these experiments were accepted for presentation at the American Society for Mass Spectrometry Conference June 1-5 in Denver and a manuscript is currently in preparation.*