

## **Biomarker Analysis of Metabolic Disorder Disease in the American Diet Mouse Model**

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**Novel Aspects:** label-free proteomics analysis of a mouse model identifies biomarkers and PTMs associated with metabolic disorder and CVD

### **Introduction:**

Unfavorable metabolic conditions (metabolic disorders) associated with obesity, diabetes, and hyperlipidemia are major causes for cardiovascular disease. One major environmental cause of this may be attributed to poor diet, aka the American diet model. The early detection and monitoring of the adverse effects of metabolic disease on the heart and vasculature, although well studied, remain elusive. Our hypothesis is that nonspecific changes which occur in plasma proteins, indicators of inflammation and oxidants, may act as evidence of systemic metabolic disease. Here we explore the application of label-free proteomics using an American diet mouse model to elucidate potential biomarkers of CVD including both protein changes and changes in observable post-translational modifications (PTMs).

### **Methods:**

Blood was from control mice and mice fed a high fat high sucrose diet (HFHS). Plasma was depleted of platelets and albumin. Proteins were obtained via precipitation and digested with trypsin. LC-MS/MS analysis was carried out on an LTQ-Orbitrap coupled with a Waters NanoAcquity HPLC. MS feature identification was enabled by analyzing the MS/MS data using Proteome Discoverer (Thermo-Fisher) and Mascot (Matrix Science) software, searching custom protein databases using both variable-modification and error-tolerant search modes. Label-free quantification was conducted using Progenesis LCMS (Nonlinear Dynamics). Collation and meta-analysis were conducted using the Trans Proteomic Pipeline (ISB), Scaffold (Proteome Software), and STRAP, STRAP-PTM (in-house) software.

### **Results:**

Label-free LCMS/MS analysis yielded over 39000 total features with over 9000 features with MS/MS ID and over 4000 features having greater than 2 fold difference. Over 790 features had ANOVAs < 0.05. Results showed excellent separation by PCA. Of interest for proteins which were up-regulated in HFHS mouse was haptoglobin, a known biomarker that becomes elevated by causative inflammation. Haptoglobin is also known to be an independent predictor of coronary vascular disorders in diabetes mellitus, which is elevated in our HFHS mouse model as early as 4 months. In addition, we saw elevations in low mannose binding protein which is associated with inflammation and cardiovascular events in type 2 diabetes, superoxide dismutase and extracellular matrix protein both implicated in type 2 diabetes which supports the use of our HFHS mouse and the many changes we see at the protein and PTM level of serum circulating proteins while on an American diet. Development of a metabolic disorder/CVD-specific protein panel obtained from these mouse models will afford the first step in biomarker panel development such that disease diagnosis and progression may be performed directly at the molecular level.

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