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Oxidative Modifications Associated with Systemic Metabolic Disorders as Early Predictors of Cardiovascular Disease

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Systemic metabolic disorders, (obesity, diabetes, hyperlipidemia) often precede cardiovascular disease (CVD). These conditions involve oxidative stress and inflammation; it seems likely that early markers for CVD could be protein PTMs resulting from metabolic irregularities. We are exploring this hypothesis by analyzing the plasma proteomes of mice maintained on a normal diet *vs*. high-fat, high-sucrose diet, with or without resveratrol treatment. A CVD-specific protein panel based on mouse models should enable development of a biomarker candidate list. Thus, the protocols are next being applied to the analysis of plasma and tissue samples from controls and from patients with metabolic disorders that underlie their CVD. Up- and down-regulated proteins and peptides are being identified using UPLC/MS/MS on the Thermo-Fisher LTQ-Orbitrap and Q-Exactive and the Bruker SolariX 12-T FTMS systems, with close attention to the detection of oxidative PTMs. We use both stable-isotope labeling and label-free proteomics approaches; abundant protein removal precedes analysis of both the abundant and low-abundance protein fractions. Isolation of abundant proteins yields ample quantities of well-defined proteins; these may bear informative makers that can serve as early indicators of disease. Low-abundance fractions are analyzed to determine which components exhibit +/- regulation and to find PTMs that may be disease markers. We are developing software to aid in the assignment and quantitative profiling of post-translationally modified proteins and peptides. Hierarchical clustering has yielded five distinct quantitative profiles. Some trends have been found to be diet-related; in the mouse study, not all responded to resveratrol treatment. MS/MS identified >200 relevant proteins. Proteins with a molecular function GO term which increased with the diet decreased with the treatment, *e.g.*, Troponin I. Analysis of OPTMs on troponin peptides in the blood correlated with the fact that hearts of HFHS fed mice show abundant OPTMs. Cysteine acylation of key proteins is being explored as a regulatory mechanism.

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