Multiplex Quantitation of Reversible Cysteine Oxidation in Mouse Heart: Effects of Catalase Overexpression and Type-2 Diabetogenic Diet

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Introduction
Diet-induced obesity is associated with metabolic heart disease (MHD). Mice fed a high fat/high sugar (HFHS) diet developed myocardial hypertrophy and diastolic dysfunction typical of MHD, in association with increased oxidative stress in the myocardium. Cardiac-specific overexpression of catalase (CatTG), an enzyme which catalyzes the decomposition of intracellular hydrogen peroxide, decreased myocardial reactive oxygen species (ROS) and prevented MHD. Using proteomics with thiol reactive iodo-Tandem Mass Tags (iodoTMT™) in a switch assay, we investigated global changes in reversible cysteine oxidation of the cardiac proteome in mice on HFHS diet and determined the effect of cardiac overexpression of catalase. Our goal is to establish multiplex quantitation approaches to investigate redox-sensitive cysteine-containing proteins and their functions in metabolic cardiovascular disease.

Methods
Left ventricles from wild-type (N=5) and CatTG trangenic FVB/N mice (N=5) fed chow or a type-2 diabetogenic diet for 8 months were used. Samples were separately lysed and labeled for total available and reversibly-oxidized cysteines with isobaric iodoTMT™ tags. Affinity-enriched TMT-tagged peptides were subjected to mass spectrometry analysis with a Q ExactiveTM mass spectrometer (Thermo Scientific). In pair-wise comparisons, ratios of reporter ions were calculated from changes in total available and reversibly-oxidized cysteines. Proteome Discoverer was applied for peptide/protein identification and quantition, using Sequest HT search against the Uniprot database. Peptides with redox-sensitive cysteines were distinguished through clustering in GProx. Proteins of interest were subjected to Ingenuity Pathway Analysis to investigate their biological significance.

Preliminary Results/Abstract
Using iodoTMT6^{plex}, tagged lysates from 5 biological replicates were combined into two groups: (A) wild-type fed chow, wild-type fed HFHS diet, and CatTG mice fed HFHS diet, and (B) wild-type fed chow, CatTG mice fed chow and CatTG mice fed HFHS diet. Changes in total available and reversibly-oxidized cysteines were compared based on ratios of reporter ions between (1) wild-type fed HFHS *vs.* chow, (2) CatTG *vs.* wild-type both fed chow, and (3) CatTG fed HFHS *vs.* chow. 658 peptides derived from 337 proteins were observed from at least two replicates with changes in reversibly-oxidized cysteines ? 30% coefficient of variation. Our results indicated that catalase overexpression caused a global 1.5- to 2- fold decrease in reversibly-oxidized cysteines in mice fed chow diet. These data suggest that H_2O_2 consumption by catalase protects cysteines from reversible oxidation in the normal heart. Peptides of interest were clustered in GProx based on reporter ion ratio with changes in cysteine oxidation altered by catalase and HFHS diet. In HFHS-fed mice, there were 47 proteins with reversible cysteine oxidization that was attenuated by catalase. IPA analysis showed that 19 of these proteins were involved in defined biological pathways including: (1) mitochondrial dysfunction: NADH dehydrogenase flavoprotein 1 (Ndufv1) and iron-sulfur protein 2 (Ndufs2) in complex I; cytochrome c oxidase (Cox6b1) in complex IV;, and carnitine palmitoyltransferase I (Cpt1b); (2) TCA cycle II: fumarate hydratase isoform (Fh); aconitate hydratase (Aco2); (3) glutathione-mediated detoxification: glutathione S-transferases (Gsto1 and Gstm1). Our work provides a systematic approach for multiplex quantitation in biological tissues to discover proteins with reversibly-oxidized cysteines that are catalase-sensitive, suggesting they are oxidized by increased H_2O_2. These proteins may be good candidates to elucidate the role of redox regulation of protein function in MHD. This research is supported by NHLBI contract HHSN268201000031C.

Novel Aspect
Multiplex quantitation of reversible cysteine oxidation in CatTG mice identifies proteins susceptible to increased H_2O_2 caused by diabetogenic diet.