SEARCH FOR SPECIFIC STRUCTURALLY ALTERED SKELETAL MUSCLE PROTEINS IN AGING SOD1$^{-/-}$ MICE: NOVEL PROTEOMIC APPROACHES

Pierce*, E. De Waal, T. Huang, H. Van Remmen, A. Richardson, A.Chaudhuri

The University of Texas Health Science Center at San Antonio, San Antonio, TX. 78229; The Sam and Ann Barshop Institute for Longevity and Aging Studies, San Antonio, Texas 78229-3900; The Geriatric Research Education and Clinical Center, Audie Murphy Memorial VA Hospital, San Antonio, Texas 78229.

Protein carbonyls are considered a sensitive marker for measuring protein oxidation in aging and age-related diseases. However, little information is available on how protein carbonylation alters protein structure and function *in vivo*. Two fluorescence-based proteomic approaches were used to identify specific proteins in skeletal muscle that had elevated protein carbonyls and altered surface hydrophobicity, a region on protein sensitive to conformational alteration. We used skeletal muscle from 12 and 24 month old wild type (WT) and antioxidant deficient SOD1 knockout (SOD1$^{-/-}$) mice to test the hypothesis that oxidation induced alteration of protein structure is observed in aging and oxidative stress. Proteins were labeled with fluorescent probes specific for carbonyls or hydrophobic surfaces and separated by 2D gel electrophoresis to identify specific proteins. Overall, a positive correlation between carbonyl levels and decrease in surface hydrophobicity was observed. Carbonyl levels were increased globally with age in WT mice by 42%, however in young and old SOD1$^{-/-}$ mice, a 38% and 48% increase was observed in skeletal muscle vs. young WT. This was accompanied by a 3, 5, and 13% decrease in global surface hydrophobicity in old WT, young and old SOD1$^{-/-}$, respectively vs. young WT. Specific proteins were identified with elevated carbonyls and altered surface hydrophobicity by mass spectrometry. Creatine kinase (CK) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) showed a substantial increase in carbonyls and decrease in surface hydrophobicity with age and genotype. Interestingly, enolase and carbonic anhydrase III (CA3) were found to be heavily carbonylated with age and genotype, however surface hydrophobicity of enolase was decreased only in old animals, and CA3 showed a 10% increase only in old SOD1$^{-/-}$ mice. Activities of identified enzymes will be measured. These data therefore, strongly suggest that oxidative damage and alteration of structure of specific proteins observed in aging and SOD1$^{-/-}$ mice are directly correlated.