

## EFFECTS OF AGING AND CALORIC RESTRICTION ON PROTEIN PROFILES IN MICE

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With the development of new technologies, the field of global gene expression (genomics) is rapidly growing, and it is now possible to measure the levels of thousands of gene transcripts in cells/tissues as a function of age. However, it cannot be assumed that changes in the mRNA transcripts reflect the changes in the levels of the protein products. In addition, post-translational modification of proteins has the potential to greatly complicate protein species expressed by an individual gene. In this presentation, we will describe the methodology we have developed to study the effect of age on the proteome of the cell using high resolution 2-D PAGE coupled with mass spectrometry. Mitochondrial protein levels were measured in liver, heart, and skeletal muscle (gastrocnemius) of 6- to 25-month-old rats fed *ad libitum* or a caloric restricted (CR) diet. Identification of the mitochondrial proteins that changed with age or CR revealed that these proteins were varied among the three tissues, i.e., no one mitochondrial protein was found to change with either age or CR in all three tissues studied. Thus, the effect of age on the mitochondrial proteome appears to be tissue-specific, and CR has only a minor effect on age-related protein changes.

We have also developed several fluorescent based assays to study the effect of age on post-translational changes in the proteome, e.g., oxidative damage (carbonyl groups and thiol/disulfides) and conformation (surface hydrophobicity). Carbonyl groups in proteins were labeled with fluorescein-5-thiosemicarbazide (FTC), and thiol and disulfide groups in specific protein were labeled with 6-iodoacetamidofluorescein (6-IAF). Age-related changes were observed in the level of carbonyl groups and thiol/disulfides in specific proteins from the liver cytosol from mice using with 2-D PAGE and MALDI-MS. For example, an age-related increase in carbonyl groups was observed in albumin, aldolase2 B, regucalcin, and Cu/ZnSOD, and an age-related decrease in disulfides were observed in selenium binding protein 2, arginosuccinate synthetase, carbonic anhydrase III, fatty acid binding protein 1, peroxyredoxin 1, and Cu/ZnSOD. A novel method was developed for screening the proteome of a cell for changes in protein conformation using the photo-incorporation of the fluorescent hydrophobic probe, BisANS, to detect surface hydrophobicity levels in specific proteins. Using this method to screen the proteome of skeletal muscle of an ALS model, we identified two proteins, creatine kinase and glyceraldehyde-3-phosphate dehydrogenase, which showed a dramatic decrease in surface hydrophobicity that correlated with the progression of disease.