

## Proteomic Characterization of Caloric Restriction and Rapamycin's Effect on Protein Aggregation in the Aging Liver

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**Background:** Impaired proteostasis is a hallmark of aging and age-related pathologies, which can be mitigated with the anti-aging interventions of caloric restriction (CR) and rapamycin (RP). Accumulation of protein aggregates such as lipofuscin, or indigestible cellular inclusions, is a typical manifestation of age-related proteostatic decline that was described over 150 years ago. Despite years of study, the composition of insoluble protein aggregates and their responses to CR and RP remains elusive.

**Methods:** Protein lysates of C57BL/6 livers were extracted from 4 cohorts (each n=6): young 4-months-old mice fed a control diet (YCL), and old 26-month-old mice that were exposed to a control diet (OCL), 40% caloric restriction (OCR), or 2.24mg/kg rapamycin (ORP), for 10 weeks. Soluble protein fractions were first separated. Detergent insoluble protein aggregates were collected by a solubilization of the remaining pellets in 8M Urea. As protein aggregates are modified with poly-ubiquitin chains, overall aggregation levels were determined with western blot analysis of poly-ubiquitin. Shotgun mass spectrometry (MS) was used to ascertain protein identities and characterize insoluble proteome abundance differences between the 4 cohorts.

**Results:** Protein aggregates were significantly higher in OCL compared to YCL ( $p = 0.02$ ). Compared to OCL, the level of poly-ubiquitinated aggregates in OCR showed a downward trend ( $p=0.059$ ). MS analysis revealed that the abundances of proteins found in the actin cytoskeleton, PKA signaling, and mitochondrial function pathways were significantly increased in OCL insoluble fraction compared to YCL. Insoluble protein abundance ratios of OCR/OCL mimicked the ratios of YCL/OCL ( $r=0.51$ ,  $p=1.29e-14$ ). Similar trends were seen when ORP/OCL abundance ratios were compared to YCL/OCL ratios ( $r=0.50$ ,  $p=2.18e-13$ ).

**Conclusion:** Age-related aggregation of proteins is not indiscriminate and can be uniquely profiled to proteins sharing similar functional pathways. Calorie restriction and rapamycin partially reverse these changes, thereby restoring the proteome to a more youthful state. Our results show that proteomic analysis of detergent insoluble protein fractions can be a useful method for identifying candidates that participate in protein aggregation and furthering our understanding of the mechanisms of proteostatic decline in aging tissues.