GLUTATHIONE, MITOCHONDRIAL DEFECTS, AND A UNIQUE METABOLIC CYCLE IN OLDER HUMANS: IMPLICATIONS FOR SARCOPENIA

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Sarcopenia in aging leads to decreased muscle mass and physical-function (muscle strength and exercise capacity), but underlying mechanisms are not well understood and effective interventions are limited. We hypothesized that deficiency of intracellular antioxidant protein Glutathione initiates a unique self-perpetuating metabolic cycle linking impaired fasting mitochondrial fuel-oxidation (fMFO) to protein catabolism and contributes to sarcopenia. We also hypothesized that supplementing the Glutathione precursor amino-acids glycine and N-acetylcysteine (GlyNAC) to correct Glutathione deficiency in older humans could reverse these defects. We tested our hypothesis in a 24-week open-label clinical-trial in 8 older-humans (74y) studied before and 24-weeks after GlyNAC supplementation, compared to 8 gender-matched unsupplemented young-controls (25y), and measured intracellular Glutathione concentrations, fMFO, physical-function, muscle-protein breakdown-rate (MPBR), gluconeogenesis, and urine nitrogen-excretion (UNE). GlyNAC supplementation in older humans corrected Glutathione deficiency and restored impaired fMFO (to levels in young controls), lowered MPBR and UNE, and increased physical-function, but did not affect gluconeogenesis or increase lean-mass, and suggest that muscle amino-acids are utilized for energy needs rather than glucose production. The absence of an increase in lean-mass suggests that GlyNAC should be combined with anabolic agents for potential benefits in combating sarcopenia. Overall, these results indicate the presence of a unique reversible metabolic cycle in older humans initiated by Glutathione deficiency which results in impaired mitochondrial fatty-acid and glucose oxidation, muscle-protein breakdown, UNE, and leads to deficiency of glycine and cysteine which re-initiate the cycle. These data have implications for improving physical-function and muscle mass in age-associated sarcopenia, and warrants further investigation.

MTOR PROMOTES BBB BREAKDOWN IN A MODEL OF ALZHEIMER’S DISEASE

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Cerebral amyloid angiopathy (CAA) is characterized by fibrillar amyloid β (Aβ) association with cerebrovasculature, which leads to impaired brain vascular function, and is present in 87% of people with Alzheimer’s disease (AD). We previously showed that inhibition of mTOR by rapamycin prevented BBB breakdown and reduced vascular fibrillar Aβ in 18-19 month old Tg2576 mice that model AD-associated CAA. This finding suggests that mTOR attenuation restores integrity of the blood brain barrier (BBB) and concomitantly reduces vascular Aβ accumulation in this mouse model. Objective: To determine the mechanisms by which mTOR drives BBB breakdown we measured the abundance of tight junction proteins zonula occludens 1 (ZO-1), occludin, and claudin-5. Methods: We used immunofluorescent confocal microscopy on frozen brain tissue sections of the same Tg2576 mice used in the previous study. Results: We confirm BBB breakdown in Tg2576 mouse brains and showed that some, but not all tight junction proteins measured were decreased in cerebrovasculature of Tg2576 mice. Attenuation of mTOR by rapamycin preserved BBB integrity, decreased vascular Aβ accumulation, and increased levels of tight junction protein abundance in Tg2576 mice, which also showed a reduced numbers of cerebral microhemorrhages. Conclusions: Taken together, these data suggest that mTOR promotes brain vascular Aβ deposition, BBB breakdown and vascular damage in the Tg2576 mouse model. Thus, mTOR inhibitors such as rapamycin – an FDA approved drug - may have promise in the treatment of AD and other dementias with related cerebrovascular dysfunction.

NOVEL STAIN SEPARATION METHOD FOR AUTOMATIC STEREOLOGY OF IMMUNOSTAINED TISSUE SECTIONS

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Many studies of brain aging and neurodegenerative disorders such as Alzheimer’s and Parkinson’s diseases require rapid counts of high signal: noise (S:N) stained brain cells such as neurons and neuroglial (microglia cells) on tissue sections. To increase throughput efficiency of this work, we have combined deep learned (DL) neural networks and computerized stereology (DL-stereology) for automatic cell counts with low error (<10%) compared to time-intensive manual counts. To date, however, this approach has been limited to sections with a single high S:N immunostain for neurons (NeuN) or microglial cells (Iba-1). The present study expands this approach to protocols that combine immunostains with counterstains, e.g., cresyl violet (CV). In our method, a stain separation technique called Sparse Non-negative Matrix Factorization (SNMF) converts a dual-stained color image to a single gray image showing only the principal immunostain. Validation testing was done using semi- and automatic stereology-based counts of sections immunostained for neurons or microglia with CV counterstaining from the neocortex of a transgenic mouse model of tauopathy (Tg4510 mouse) and controls. Cell count results with principal stain gray images show an average error rate of 16.78% and 28.47% for the semi-automatic approach and 8.51% and 9.36% for the fully-automatic DL-stereology approach for neurons and microglia, respectively, as compared to manual cell counts (ground truth). This work indicates that stain separation by SNMF can support high throughput, fully automatic DL-stereology based counts of neurons and microglia on counterstained tissue sections.

SOLUBLE INTERCELLULAR ADHESION MOLECULE (SICAM-1) AS A BIOMARKER OF VASCULAR COGNITIVE IMPAIRMENT IN OLDER ADULTS

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Background: Endothelial dysfunction and subsequent inflammation contribute to the development of vascular cognitive impairment (VCI). Soluble intercellular adhesion molecule-1 (sICAM-1) is upregulated in endothelial dysfunction and promotes an inflammatory response; however, the relationship between sICAM-1 and VCI remains equivocal. Objective: To determine whether sICAM-1 contributes to the prediction of VCI. Methods: Community-dwelling older adults (n=172) from the “Cohort of Obesity, Sarcopenia and Frailty of Older Mexican Adults” (COSFOMA) study were identified as VCI or controls using standard neuropsychological evaluations and neuroimaging. sICAM-1 was quantified using ELISA, and multivariate logistic regression determined the association between sICAM-1 and VCI. Results: 31 VCI cases were identified. sICAM-1 was higher in VCI [VCI: 450.7 (241.6) ng/ml vs. Control: 296.9 (140.9) ng/ml]. sICAM-1 concentrations above the 90th percentile (464.1 ng/mL) was associated with VCI group membership in all models [OR = 6.9 (95% CI: 1.1-42.2)]. The final saturated model explained 64% of the variance in VCI group membership. Conclusion: High concentrations of sICAM-1 are independently associated with VCI group membership. Efforts to further characterize the relationship between indices of endothelial dysfunction and pathological changes to the aging brain should be further pursued.

SINGLE-CELL TRANSCRIPTOMICS OF AGING MOUSE ISLET REVEALS AGE-RELATED RECRUITMENT OF ISLET-RESIDENT MACROPHAGE
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Type 2 diabetes (T2D) prevalence increases with age. The notion of inevitable progression of T2D has been challenged by reports of remission in some human T2D cases; however, this remission is dependent on islet function reserve. To elucidate the molecular mechanisms driving islet cell dysfunction, it is necessary to understand islet cell composition, diversity, and function throughout the lifespan. We generated a single-cell transcriptomic atlas of healthy islets isolated from young (5 weeks old), middle-aged (12 months old), and older-aged (25 months old) mice. Cell clustering identified 13 initial cell clusters that were further sub-clustered. This single-cell RNAseq profile showed that each cell type/group has different markers and functional characteristics and that age causes a remarkable shift in islet cell composition, diversity, and number. By comparing macrophages from young and old mice, we also found that aged islets contain a higher number of islet-resident macrophages. Overall, this single-cell islet atlas covers nearly all cells in the normal islet and allows a comprehensive exploration of all transcriptional states throughout the lifespan.

ASSESSING THE RELATIONSHIP BETWEEN SERUM IGF-1 AND ADIPOSITY BY AGE IN THE LONG LIFE FAMILY STUDY
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Serum levels of insulin-like growth factor 1 (IGF-1) and measures of adiposity, such as body mass index (BMI), are associated with susceptibility to age-related diseases. Previous reports of the relationship between IGF-1 and BMI ranged from positive to negative to no relationship, perhaps because previous reports studied different age cohorts. Using data on 4270 participants (aged 24-110 years) from the Long Life Family Study, we investigated the relationship between IGF-1 and BMI overall and by age groups. IGF-1 and BMI were positively correlated in the total sample (β=0.161, r²= 0.0038, p=1.8-05). However, further analyses revealed that the relationship between IGF-1 and BMI varied by age quartile: in the 1st quartile (24-58yo) the relationship was negative (β=-0.204, r²= 0.011, p=0.0008); in the 2nd quartile (59-66yo) the relationship was positive and non-significant (β=0.069, r²= 0.0012, p=0.28); in the 3rd quartile (67-86yo) the relationship was positive but non-significant (β=0.106, r²= 0.002, p=0.13); and in the 4th quartile (87-110yo) the relationship was positive (β=0.388, r²= 0.019, p=1.2-05). This pattern did not differ by sex. We also detected a similar age-related pattern between IGF-1 and BMI using an independent dataset (NHANES III), comprising 2550 men and women aged 20-90 years. Our results may clarify some of the inconsistency in previous literature about the relationship between IGF-1 and BMI. Additional studies of IGF-1 and adiposity measures are needed to better understand the underlying mechanisms involved.

FASTING-MIMICKING DIET REDUCES RISK FACTORS FOR AGING-RELATED DISEASES IN PRECLINICAL AND CLINICAL STUDIES
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Prolonged fasting promotes stress resistance, but its effects on longevity are poorly understood. Calorie restriction or major dietary composition changes can have profound effects on healthy aging but the inability of many subjects to adhere to chronic and extreme diets together with the potential of adverse effects limit their application. Fasting-mimicking diets (FMDs) are effective in increasing health and lifespan, possibly by inducing stem cell-based regeneration, or as therapies in mouse models of a variety of diseases. FMDs reduce cancer incidence/progression, modulate the immune response, reduce immuno-senescence, ameliorate or reverse disease progression of multiple sclerosis, Type I and Type II diabetes, and reverse inflammatory bowel disease pathology. In a randomized clinical trial, markers/risk factors for metabolic syndrome and other age-related diseases were favorably impacted after completion of 3 FMD cycles. These effects were larger in participants at risk for age-related diseases.