Gerontology and studies of aging have been grappling with the effects of a westernized diet for decades now. Some of these effects include diseases related to over-consumption such as obesity, diabetes, hypertension, cardiovascular disease, stroke, and many more. These pathologies are now contributors to many of the leading causes of death, with humans craving much more salt than is necessary for physiological homeostasis. Our relationship with salt is already unhealthy but the continuation of our current diets will be passed on to the next generation with unforeseen consequences.

To analyze the effects of high salt on health span and aging, we used *Drosophila melanogaster* and genetic manipulation to show that a high salt diet is dehydrating and can be rescued through addition of water or changing expression in a key, regulatory protein: *Locomotion defect* (*Loco*). While this has been shown in young flies before, we confirmed this finding and extended our study to older flies of 3 and 5 weeks of age. The pertinence of chronic kidney disease, the high salt pandemic, and inability to regulate ion balance with age contribute to the motivation to explore a genetic mutation of *Loco* and why deficiency in this protein and its associated G-Protein signaling pathway confers resistance to high salt stress. Additionally, extensive literature review and exploration of related insulin signaling/cAMP pathways illuminates a possible link between high salt, hypertension, oxidative stress, and the current theory of molecular aging and senescence.

*Loco* has an orthologous RGS in the mammalian kidney, which means insights in the Drosophila system may translate into future mammalian research. In a critical study, a research group at the New Jersey Medical School under expressed and overexpressed *Loco*. The overexpression resulted in significantly reduced lifespan. The reduced expression resulted in a preservation of life response with a longer lifespan and stronger resistance to heat, starvation, and oxidation. The reduction also resulted in higher fat content and higher manganese-containing superoxide dismutase (MnSOD) expression. Additionally, the study considered the homologous yeast and mammalian proteins Rgs2 and RGS14. The yeast demonstrated increased lifespan and stress resistance, while the mice exhibited greater stress resistance as well. This study proposed that these RGS proteins, as upstream regulators of signaling pathways associated with longevity and stress resistance, have a significant impact on lifespan across multiple organisms. More recently, our group showed that the heterozygous *Loco* mutant exhibits greater lifespan on a high salt diet.
Figure 1 – High Salt Survival +H₂O Assay

Data shown as probability of survival vs. days elapsed. 25 days shown here with solid lines indicating water being provided and dashed lines indicating the no water treatment. All groups fed a 0.3 M high salt diet; statistical analyses using a log-rank (Mantel-Cox) curve comparison *, p<0.0332; **, p<0.0021; ***, p<0.0003; ****, p<0.0001 after Bonferroni correction.

Figure 2 – Female Water Weight

Data separated into age groups and shown as mean water weights, +/- standard deviation. Loco²y-P283 mutant is represented by red, while the control is represented in gray. Statistical analyses using a 2-way ANOVA with Sidak’s multiple comparison testing. *, p<0.0332; **, p<0.0021; ***, p<0.0003; ****, p<0.0001
Aside from increased fat content and decreased cAMP signaling, there is also a known 74% increase in Manganese-containing Superoxide Dismutase (MnSOD) expression for Loco mutants. This enzyme is an antioxidant responsible for destroying superoxide, a harmful reactive oxygen species (ROS). ROS are capable of destroying many strong bonds, permeabilizing membranes, and instigating apoptotic cell death. These small molecules are often byproducts of oxidative phosphorylation and metabolism, but can also be found as part of the immune system.\(^9,10\) The ROS are microbicidal and can be secreted into the gut during infection to kill the pathogen.\(^11\) These ROS are a necessary evil, but they are still harmful to the host organism. In the presence of a resistant microbe or in an aged fly where the immune system is less effective, ROS are chronically produced and contribute to intestinal permeability. Both intestinal permeability and dysregulation of the ROS/antioxidant balance serve as markers for senescence and aging.\(^10\) The Loco mutant, known to live longer and survive greater oxidative stress, has increased expression of MnSOD.\(^7\)

How does this relate to increased durability under salt stress? It is possible that increased sodium intake may be related to an increase in ROS and subsequent oxidative stress in Drosophila. Certainly, it has been shown a high salt diet induces oxidative stress in mice through upregulation of renal ROS generation and decreased expression of intracellular and mitochondrial MnSOD.\(^12\) Even in humans, enhanced production of reactive oxygen species has been linked to certain diseases of the cardiovascular system including hypertension and diabetes.\(^13-15\) Hypertensive mammalian models have shown that chronic treatment with antioxidant drugs could completely prevent or markedly attenuate hypertension.\(^14\) There is still much to be tested especially in Drosophila, but it seems that oxidative stress, salt stress, and hypertension may be related in some way. The increased antioxidant capacity in Loco mutants is certainly a promising target for future testing and manipulation, especially in the context of a high salt diet.

Although our understanding of Loco is still far from complete, there are connections to more understood pathways such as insulin signaling, oxidative stress, and transcription factors like dFOXO. We have shown in the salt survival and water weight assays that a high salt diet produces dehydration stress in Drosophila melanogaster. Loco\(^{E(Y)=P283}\) mutants have a greater ability to survive this dehydration stress because they can retain more of their initial water content, which is not significantly different than controls. This, in combination with the previously known longevity and stress resistance phenotypes, has led us to believe there is a complex, multi-faceted change in gene expression induced by Loco deficiency. Either through preventative mechanisms, enhanced ability to repair damage from ROS, or some combination of both, the Locomotion defect RGS poses an exciting possibility as a single-target manipulation for health span extension even in the presence of excess salt.


