

O F A S

Life's blood flows through the hourglass; the stopcock represents the alteration of aging and disease as biomedical research progresses.

Report of the Director

Dear Friends,

In 2024, the Orentreich Foundation for the Advancement of Science continued its research endeavors in ways to extend lifespan and healthspan in both animals and humans. Our scientists have achieved international recognition for their studies, which range from molecular and genetic level examinations to whole animal disease models, with promising results.

We also continued our commitment to support promising young investigators by awarding the Norman Orentreich Young Investigator Award to Dr. Gregor Bieri, a postdoctoral fellow at University of California at San Francisco, for his groundbreaking research explaining the biochemical signaling by which exercise chemically signals and modulates the brain, resulting in enhanced neural function in aging.

This report presents the findings of our scientists' investigation into the mechanisms of healthspan extension produced by dietary sulfur amino acid restriction (SAAR). These studies have unveiled novel and more accessible approaches to achieving the benefits of SAAR without the difficulties of trying to adhere to a diet low in sulfur amino acids. Concurrently, these interventions revealed that it was possible to eliminate the few known negative effects of SAAR: a slight loss of lean body mass and a mild reduction in bone strength.

In studying the mechanisms by which SAAR benefits are conferred on cells, one of our investigators has found a drug that promises a treatment for a genetic form of diabetes characterized by defective insulin folding into its active conformation. In addition, our previous research on the neurodegenerative processes associated with amyotrophic lateral sclerosis (ALS, or Lou Gehrig's Disease) have been extended to the study of Parkinson's disease in 2024.

These significant research endeavors would not have been possible without your unwavering support and faith in our mission. We are grateful for your continued support.



David S. Orentreich, MD, FAAD
CEO



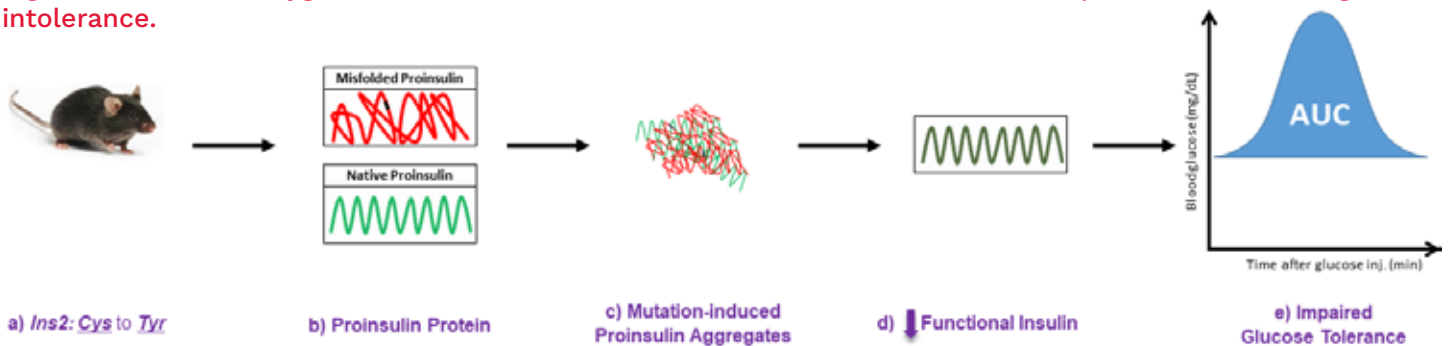
Nichenametla Laboratory

Dr. Nichenametla's team discovered that D,L-buthionine sulfoximine (BSO), a drug previously used in clinical trials for cancer treatment, also treats a specific type of diabetes, which occurs due to a mutation in the insulin gene. Akita mice harbor a mutation in the *Ins2* gene, leading to proinsulin misfolding. Consequently, misfolded proinsulin molecules interfere with wild-type proinsulin folding, leading to diminished functional insulin and impaired glucose regulation. These mice exhibit a 3- to 4-fold elevation in blood glucose compared to mice without the mutation. Dr. Nichenametla hypothesized that administering BSO to these mice would enhance cellular mechanisms that prevent the mutated proinsulin molecules from interfering with the wild-type proinsulin molecules. His team's finding revealed that Akita mice treated with BSO exhibited lower blood glucose levels and improved glucose tolerance compared to Akita mice not treated with BSO. They hypothesize that BSO's effects may be mediated by targeting cellular processes regulating protein quality and the local oxidative balance. These findings suggest that BSO could be a promising therapeutic option for individuals with Mutant Insulin Diabetes of Young, a genetic condition similar to that found in Akita mice.



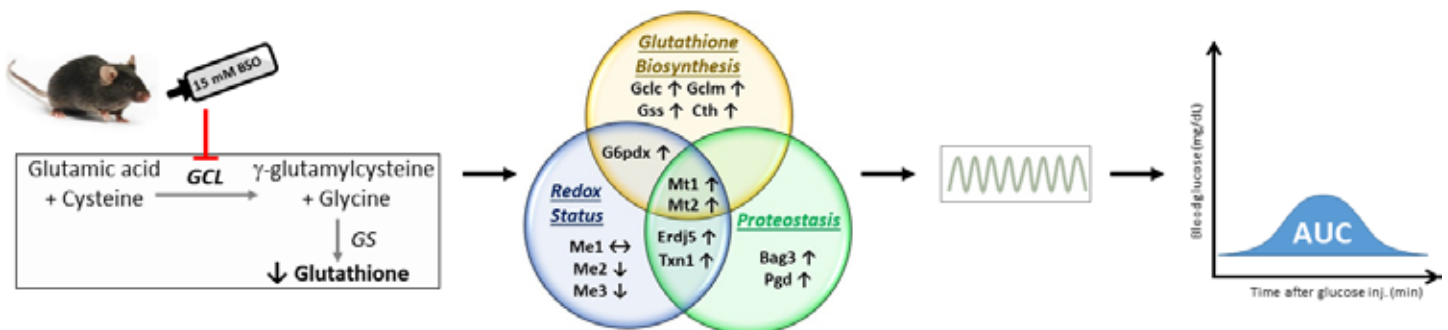
Sailendra Nichenametla, Ph.D.
Senior Scientist

Figure 1. Male heterozygous C57BL/6-*Ins2*Akita/J (AK) mice suffer from misfolded proinsulin-induced glucose intolerance.



(a) Proinsulin misfolding occurs due to a genetic mutation in *Ins2* gene that substitutes Cys with Tyr, (b) Due to heterozygosity, AK mice produce both wild-type and mutated proinsulin, (c) Mutated proinsulin forms aggregates with itself and with the bystander native proinsulin, (d) Proinsulin aggregation results in lower functional insulin, and (e) AK mice suffer from impaired glucose tolerance.

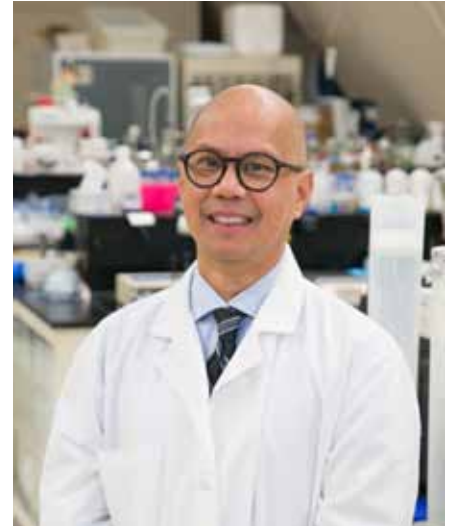
Figure 2. Treating mice with BSO improved glucose tolerance.



(a) Mice were treated with continuous administration of 15 mM DL -buthionine-(S,R)-sulfoximine (BSO), an inhibitor of glutathione biosynthesis (b), BSO treatment increased the renal mRNA quantity of several genes involved in glutathione biosynthesis, glutathione redox status, and proteostasis, (c) we hypothesize that BSO-induced changes in cellular redox status and gene expression ameliorates proinsulin aggregation and increases the functional insulin levels in β -cells, and (d) BSO treatment significantly improved glucose intolerance in AK mice. Note: AUC: Area under the curve, GCL: γ -g-glutamylcysteine ligase, GS: Glutathione synthetase.

As the most prevalent movement disorder in humans, Parkinson's disease (PD) is characterized by the loss of dopaminergic neurons and the accumulation of α -synuclein accumulation in the brain. Currently there is no cure; existing treatments focus on alleviating symptoms by increasing dopamine levels. The effect of SAAR on neurodegenerative diseases, including PD, remains largely unexplored. Consequently, we have initiated a preliminary study employing a mouse model of Parkinson's, specifically the human α -synuclein transgenic A53T mice (A53T Tg), to investigate the progression of the disease. We hypothesize that SAAR will delay disease progression in the mouse model by reducing dopamine loss, enhancing mitochondrial activity and autophagy, and mitigating oxidative stress and neuroinflammation, subsequently diminishing α -synuclein accumulation.

In addition, we continue to study the effects of a high-fat SAAR diet in a mouse model of amyotrophic lateral sclerosis (ALS). Previously, we demonstrated that a low-fat SAAR diet delayed ALS symptom onset in a mouse model (SOD1-G93A) but decreased overall survival compared to control mice. We speculate that the shortened survival in low fat SAAR-SOD1 mice may be attributed to: (1) early energy depletion as the mice attempt to delay disease progression, (2) the need for a less stringent SAAR diet to maintain body mass, or (3) SAAR activation of protective pathways may become detrimental over time. We hypothesize that a high-fat SAAR diet will delay disease progression and prevent shortened survival in the SOD1 mice.



*Gene Ables, Ph.D.
Associate Research Scientist*

Other ongoing projects include:

- A follow-up study in collaboration with Drs. Mark Horowitz, Basav Ganganna, and Doug Rothman at Yale University to evaluate the suitability of high-resolution MRI for measuring adipocyte depots, particularly bone marrow fat, in mice.
- A collaboration with Drs. Calvin Vary, Robert Koza, Rea Anunciado-Koza, Marissa McGilvrey and Lucy Liaw of MaineHealth Institute for Research to examine the roles of different adipose tissue depots that are affected by SAAR.
- Studies involving SAAR with Dr. Andrey Parkhitko of the University of Pittsburgh and Dr. Tom Hampton of Mouse Specifics, Inc.

A sustained state of SAAR produces multiple metabolic health benefits and extends the healthy lifespan of several model organisms, including rodents (Ables & Johnson, 2017 *Exp Gerontol* 94: 83). For example, continuously SAA-restricted rats have less age-related pathology and are up to 45% longer-lived than their control-fed counterparts. Given that the vegan diet is low in both protein and free amino acids, SAAR is feasible for humans and studies have reported that SAA-restricted human subjects receive similar benefits to rodents. However, long-term adherence to continuous SAAR is likely to be challenging (if not impossible) for many individuals. Another obstacle to the successful translation of this intervention to human subjects is the fact that several deleterious effects of continuous SAAR have been reported for both rodents and humans. These include the loss of musculoskeletal mass, increased bone marrow adipogenesis, and an increased incidence of bone fractures. To address these issues, the Johnson Lab previously developed two novel dietary interventions that are more practicable than continuous SAAR, yet produce similar health benefits to the classical

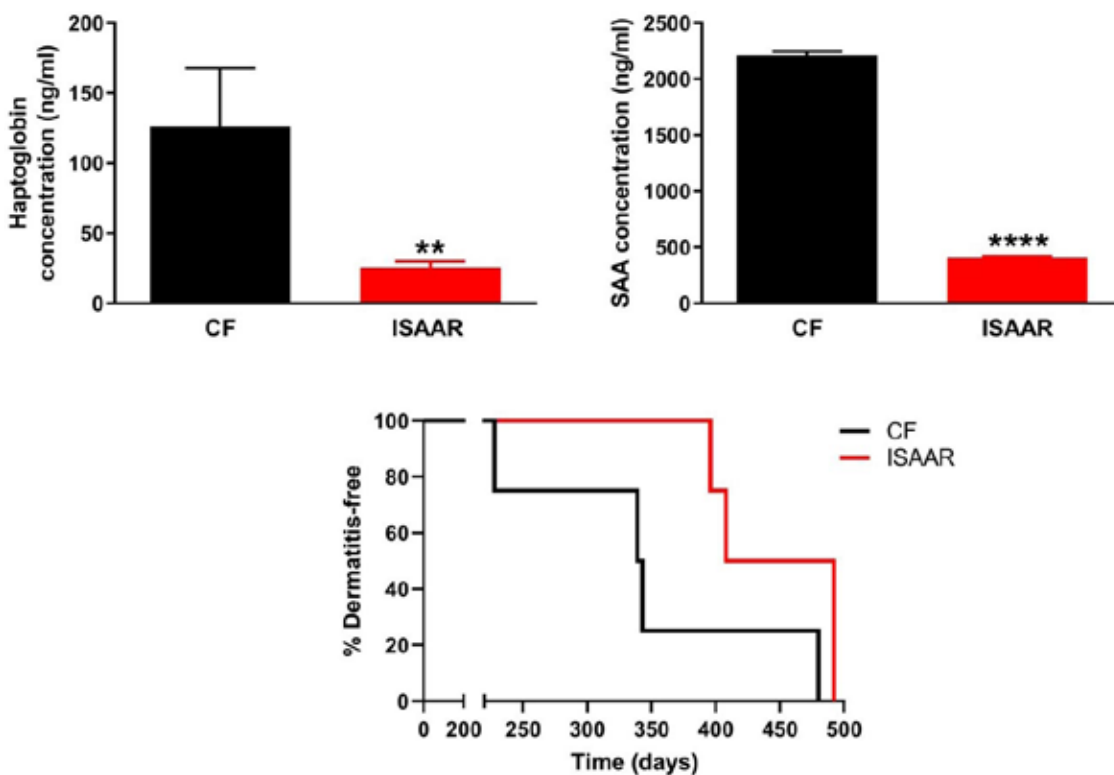
intervention. Further, these interventions cause no deleterious side effects. The first intervention involves supplementation of an otherwise normal diet with selenium (Plummer et al., 2021 *ELife* 10). The latter intervention is an intermittent form of SAAR that requires only 3 days of reduced SAA intake per week (Plummer & Johnson, 2022 *Aging Cell* e13629).



Jay Johnson, Ph.D.
Associate Research Scientist

As the role of chronic inflammation in the development of multiple age-related pathologies becomes increasingly clear, Dr. Johnson's group considered the possibility

Figure 1. Intermittent SAAR Prevents Inflammation and Protects Against the Development of Dermatitis

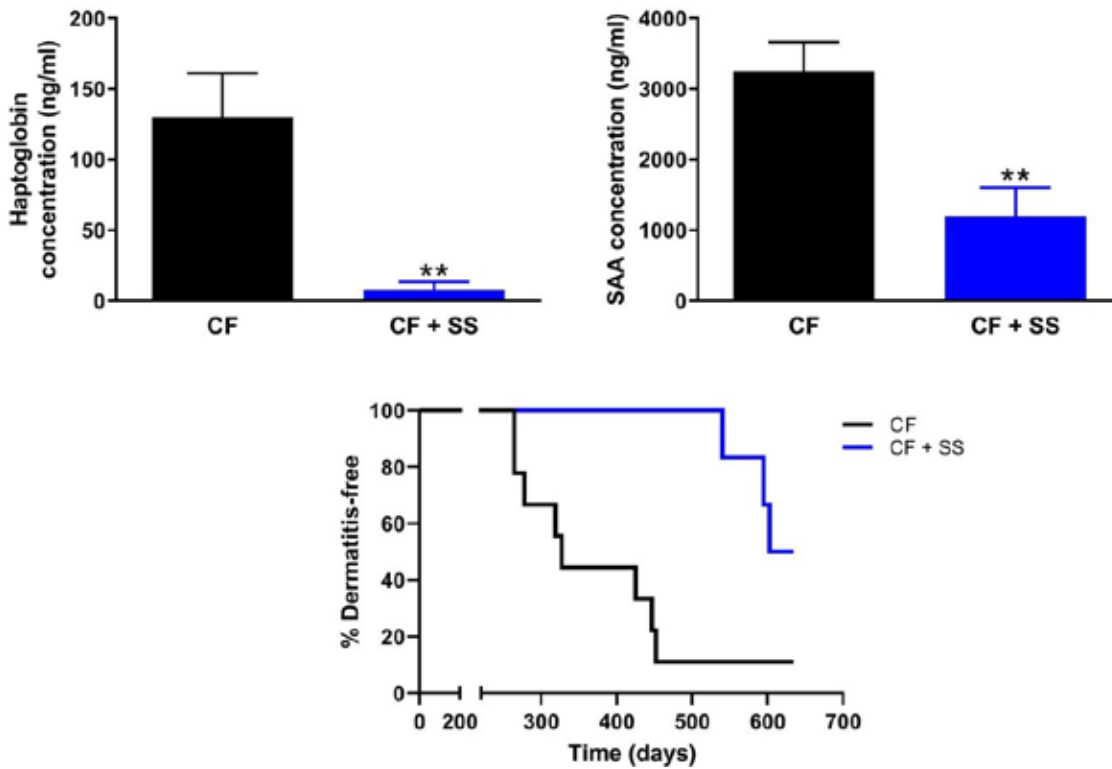


Shown are the levels of inflammation markers haptoglobin and serum amyloid A (SAA) in the plasma of control-fed (CF) and intermittently SAA-restricted (ISAAR) mice, as well as the incidence of dermatitis in these animals over the course of the experiment. For the top panels, bars denote standard error of the mean (SEM) and statistically significant differences are indicated (**, $p < 0.01$; ****, $p < 0.0001$).

that the manifold health benefits of intermittent SAAR and selenium supplementation might result, at least in part, from the prevention and/or amelioration of diet-induced inflammation. To test this hypothesis, the group assessed the levels of multiple markers of inflammation in animals fed an unhealthy high-fat diet, and observed that both intermittent SAAR and selenium supplementation protects against inflammation (Figures 1 & 2). Interestingly, both interventions also robustly protect against the development of inflammation-induced dermatitic skin lesions.

To facilitate the development of easily practicable interventions that extend healthy lifespan, the Johnson Laboratory has identified several additional compounds that exhibit SAAR-like benefits, albeit in the context of a typical, SAA-replete diet. For example, animals supplemented with any one of four specific amino acids exhibit complete protection against diet-induced obesity, (Figure 3, page 6) and demonstrate similar beneficial plasma hormone changes to SAA-restricted mice. Subsequent experiments suggest that elevated levels of these amino acids produce a signal of underfeeding, which in turn beneficially alters metabolism.

Figure 2. Selenium Supplementation Prevents Inflammation and Protects Against the Development of Dermatitis

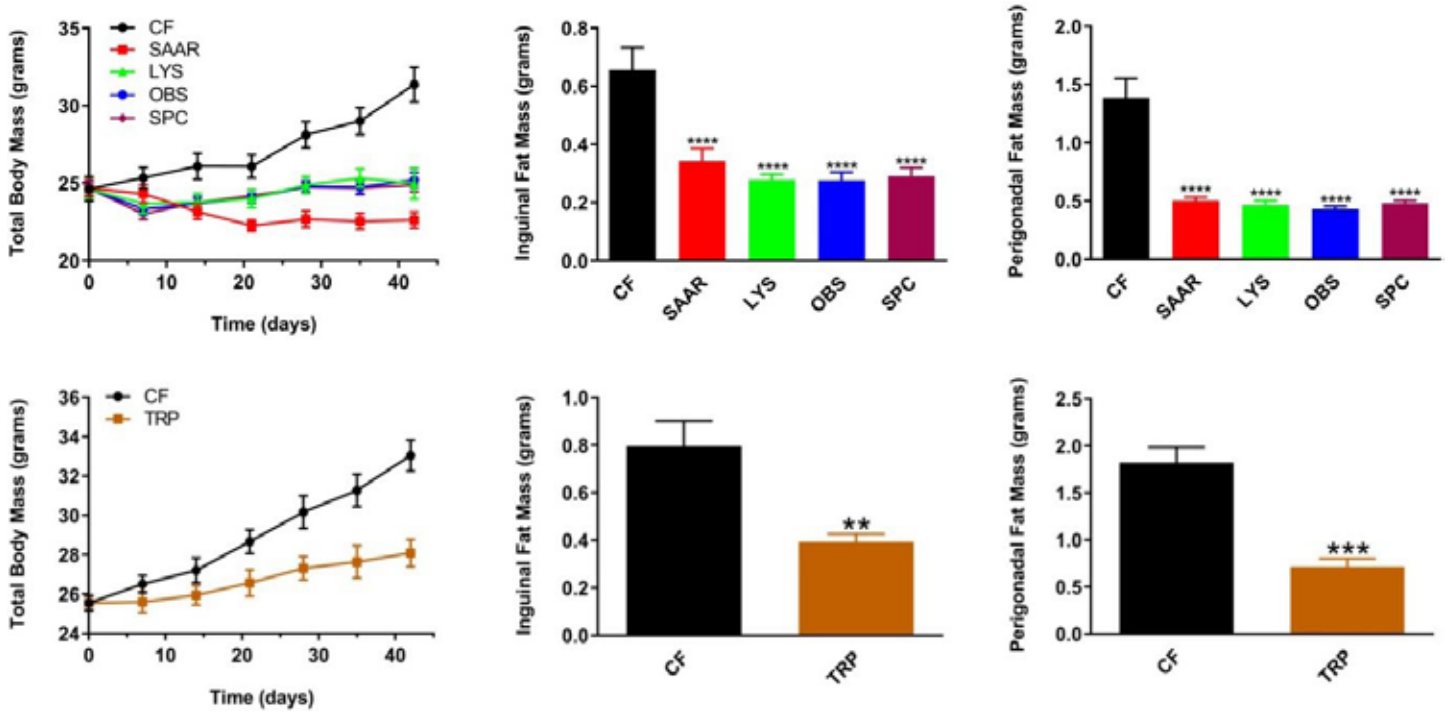


Shown are the levels of inflammation markers haptoglobin and serum amyloid A (SAA) in the plasma of control-fed (CF) and selenium-supplemented (CF + SS) mice, as well as the incidence of dermatitis in these animals over the course of the experiment. For the top panels, bars denote SEM and statistically significant differences are indicated (**, $p < 0.01$).

In summary, the Johnson Lab's recent findings have unveiled multiple novel dietary interventions that enhance the healthy lifespan of mice, demonstrating significantly greater practicability (and reduced adverse effects) as compared to continuous SAAR. Dr. Johnson

envisioned that the potential translation of some (if not all) of these promising new interventions to humans thereby improving health and mitigating the burden of age-related diseases.

Figure 3. Supplementation with Various Amino Acids Protects Against Diet-Induced Obesity



Shown are comparisons over time of average values for the total body mass of control-fed (CF) and continuously SAA-restricted (SAAR) mice, as well as animals fed otherwise normal diets supplemented with lysine (LYS), O-benzyl serine (OBS), S-phenyl cysteine (SPC), or tryptophan (TRP). Average values at the conclusion of the experiment are also shown for the inguinal and perigonadal fat mass of animals fed the various diets. Bars denote SEM for all panels. For fat mass measurements, statistically significant differences (as compared with the corresponding CF values) are indicated (**, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$).

Publications & Presentations

Plummer JD, Horowitz MC, Johnson JE. Intermittent methionine restriction reduces marrow fat accumulation and preserves more bone mass than continuous methionine restriction. *Aging Biology*, 2: 20230019, 2024.

Anunciado-Koza RVP, Yin H, Bilodeau CL, Cooke D, Ables GP, Ryzhov S, Koza RA. Interindividual differences of dietary fat-inducible Mest in white adipose tissue of C57BL/6J mice are not heritable. *Obesity (Silver Spring)*, Jun;32(6):1144-1155, 2024.

Mattocks DAL, Ommi NB, Malloy VL, Nichenametla SN. An antireductant approach ameliorates misfolded proinsulin-induced hyperglycemia and glucose intolerance in male Akita mice, *GeroScience*, 2024. <https://doi.org/10.1007/s11357-024-01326-6>.

Johnson JE. Novel amino acid-related interventions that improve the healthspan of mice (talk). Biology of Healthy Aging Seminar Series, Johns Hopkins University (Baltimore, Md.), February 13, 2024.

Ommi NB, Mattocks DAL, Nichenametla SN. DL-Buthioninesulfoximine induces lean phenotype in male diet-induced obese mice (poster). Venue: Paul F. Glenn/Afar Conference on The Biology of Aging, Santa Barbara, Calif., May 29–31, 2024.

Nichenametla, SN. The role of cysteine and serine in sulfur amino acid restriction-induced metabolic health benefits (talk). 16th International Symposium on the Neurobiology and Neuroendocrinology of Aging, Bregenz, Austria, July 15, 2024.

Cooke C, Izquierdo B, Ruseskas J, Lisikatos M, Tucker T, Hampton T, Ables GP. The impact of dietary sulfur amino acid restriction in low-fat and high-fat diets on amyotrophic lateral sclerosis using B6.SOD1-G93A mice (poster). Gordon Research Conference Neurobiology of Brain Disorders, Barcelona, Spain, August 4–9, 2024.

Johnson JE. Novel amino acid-related interventions that reduce inflammation and improve the healthspan of mice (poster). Mechanisms of Aging 2024, Cold Spring Harbor Laboratories, Cold Spring Harbor, N.Y., September 24–28, 2024.

Norman Orentreich Award for Young Investigator on Aging

OFAS presented the 2024 Dr. Norman Orentreich Award for Young Investigator on Aging to Gregor Bieri, Ph.D. Dr. Bieri is currently a Postdoctoral Scholar at the University of California San Francisco, where his work focuses on the exercise-induced liver factor Gpld1 and its role in transferring the benefits of exercise on the brain in aging and neurodegeneration. The prize was presented at the 16th International Symposium on Neurobiology and Neuroendocrinology of Aging (Bregenz, Austria) in July. With this award, we hope to inspire young investigators to continue aging research and to acknowledge the potential of their work. In addition to the \$1,000 prize, recipients are invited to present at a future OFAS Symposium.

Information for Donors

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