OFAS

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,

The Orentreich Foundation for the Advancement of Science continues to investigate ways to increase human healthspan and lifespan. Our scientists have made major inroads into understanding the role of reduced intake of sulfur amino acids (SAAR) in extending life expectancy and slowing the onset of many age-related diseases. Our research spans the gamut from molecular biology to whole animal physiology. Recent work described in this report includes SAAR-related slowing of symptom onset in amyotrophic lateral sclerosis (ALS, or Lou Gehrig's Disease), changing protein structure and processing, and even developing novel and non-dietary methods to achieve the SAAR effect.

Our founder, Dr. Norman Orentreich, was deeply committed to training young scientists. Today we continue that commitment. In 2022 we supported the American Aging Association 50th Annual Meeting (San Antonio, Tex.) and the 15th International Symposium on Neurobiology and Neuroendocrinology of Aging (Bregenz, Austria). At each meeting, we awarded the Norman Orentreich Award for Young Investigator on Aging. The recipients will be invited to a future OFAS Symposium to present updates on their careers and research. We have also undertaken to provide advanced training to an early-career researcher, Dr. Naidu Ommi, under the direction of Associate Research Scientist Dr. Sailendra Nichenamelta.

OFAS scientists often collaborate with outside investigators to increase our scientific capabilities as well as our visibility in the scientific community. Reflecting both this increased visibility and his own research talents, Dr. Nichenametla was the 2022 recipient of the American Society of Nutrition's 2022 Vernon Young International Award for Amino Acid Research.

On behalf of the entire staff at OFAS we thank you for your support, faith, and interest in our mission. We wish you a healthy and happy 2023.

David S. Orentreich, MD, FAAD
Director

Johnson Laboratory

A sustained state of sulfur amino acid restriction (SAAR) dramatically extends the healthspan of several model organisms (Ables and Johnson, 2017 *Exp Gerontol* 94: 83). For example, continuously SAA-restricted rodents have less age-related pathology and are up to 45% longer-lived than control-fed littermates. Given that the vegan diet is low in both protein and free amino acids, eating a SAA-restricted diet is feasible for humans and, promisingly, preliminary studies have suggested that SAA-restricted individuals may receive similar benefits to rodents. Unfortunately, long-term adherence to a SAA-restricted diet is likely to be challenging for some, and might be undesirable for others. Accordingly, a key goal of the aging field has been to develop simpler and/or more practicable interventions that produce healthspan benefits similar to those engendered by SAAR.

As a result, Dr. Johnson's research has focused on two goals: 1) characterizing the mechanisms underlying the benefits of SAAR, and 2) identifying novel SAAR-like interventions that improve mammalian healthspan. For this purpose, Dr. Johnson's group makes use of multiple experimental model systems, including baker's yeast, cultured mouse and human cells, and laboratory mice.

With respect to the first goal, Dr. Johnson's group has demonstrated that mitophagy (a process that selectively recycles mitochondria) is indispensable for SAAR-dependent yeast lifespan extension (Plummer and Johnson, 2019 *Front Cell Dev Biol* 7: 301). Furthermore, they have discovered that, similar to SAA-restricted animals, yeast cells undergoing SAAR demonstrate a significantly altered pattern of carbon metabolism as compared with controls.

Jay Johnson, Ph.D.
Associate Research Scientist

With respect to the second goal, Dr. Johnson has recently developed a novel form of SAAR, intermittent SAAR, that is both highly effective and also free from the disadvantages of the classical intervention (Plummer and Johnson, 2022 *Aging Cell* 6: e13629). Intermittent

Figure 1

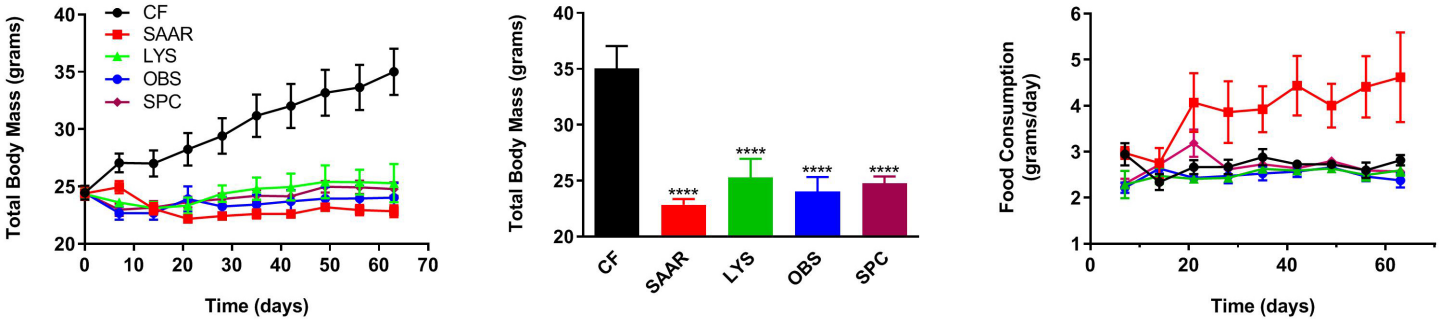
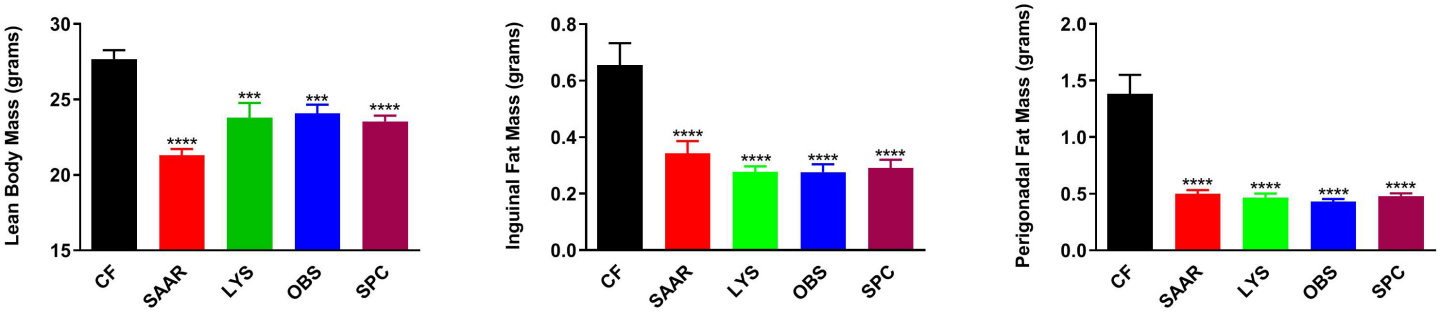


Figure 2



Johnson Laboratory

SAAR requires only 3 days per week of reduced SAA intake, yet improves glucose metabolism and insulin sensitivity, prevents fatty liver disease, and completely protects mice against diet-induced obesity. Similar to the continuous intervention, intermittent SAAR also confers beneficial changes in the levels of multiple hormones involved in the regulation of metabolism, health, and longevity. However, a notable difference between the two interventions is that, in contrast to classical SAAR, the novel intervention results in little to no growth inhibition and does not negatively impact the development of lean body mass. Relatedly, ongoing studies in the laboratory have found that animals undergoing intermittent SAAR have both stronger and healthier bones as compared with continuously SAA-restricted controls.

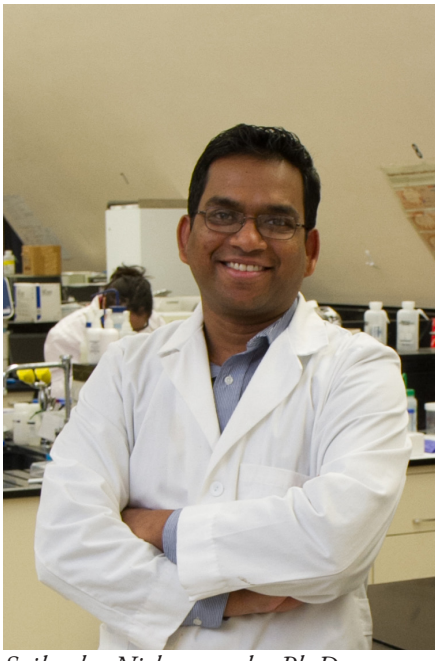
Dr. Johnson has also identified several compounds that produce SAAR-like health benefits, even in the context of a normal (i.e., methionine-replete) diet. Similar to intermittent SAAR, supplementation with these compounds protects mice against obesity and confers many other healthspan benefits. Two of these compounds contain the essential micronutrient selenium, and the benefits of their supplementation have been described (Plummer et al., *eLife* 2021, 10: e62483). Recent work in Dr. Johnson's laboratory has focused on elucidating the mechanisms underlying the benefits of several other compounds that do not contain selenium, yet also act as mimetics of SAAR. For example, supplementation of an otherwise normal mouse diet (CF) with the amino acids lysine (LYS), O-benzyl serine (OBS), or S-phenyl cysteine (SPC) protects mice against the overall weight gain (Figure 1) and accumulation of fat (Figure 2) that are ordinarily caused by consumption of a high-fat diet.

Currently, the group is assessing to what extent these interventions reduce the rate of aging in mice. It is Dr. Johnson's hope that these novel interventions can eventually be translated to humans in order to improve health and reduce the burden of age-related disease.

Nichenametla Laboratory

Observational studies in humans show that excess intake of the sulfur amino acids methionine (Met) and cysteine (Cys) is associated with obesity and other metabolic diseases. Supporting this finding, animal studies show that restricting the dietary intake of Met and Cys (sulfur amino acid restriction; SAAR), results in weight loss. The significant effect of the SAAR diet in reducing body fat in animal studies prompted similar interventional studies in humans; however, the effects of the SAAR diet in humans were not as dramatic. To improve the degree of SAAR-induced benefits in humans, it is essential to understand the mechanisms by which it decreases body fat in animals and the reasons for its attenuated effects in humans. Dr. Nichenametla's lab delineated a potential mechanism by which the SAAR diet induces weight loss in rodents.

Although both Met and Cys are essential for health, dietary intake of Cys is not essential since animals and humans can make Cys from Met. For this reason, most animal studies formulated SAAR diets with low levels of Met, and eliminated Cys. To date, not enough attention has been paid to the absence of Cys in the SAAR diet. Although animals can make Cys from Met, due to the low levels of Met in the SAAR diet, they cannot make enough of it. This implies that animals on SAAR diets undergo both methionine restriction (MetR) and cysteine restriction (CysR). Conversely, in human SAAR studies, Met was decreased but Cys was not eliminated. Dr. Nichenametla questioned whether the attenuation of the effects of the SAAR diets in humans was due to the



Sailendra Nichenametla, Ph.D.
Associate Research Scientist

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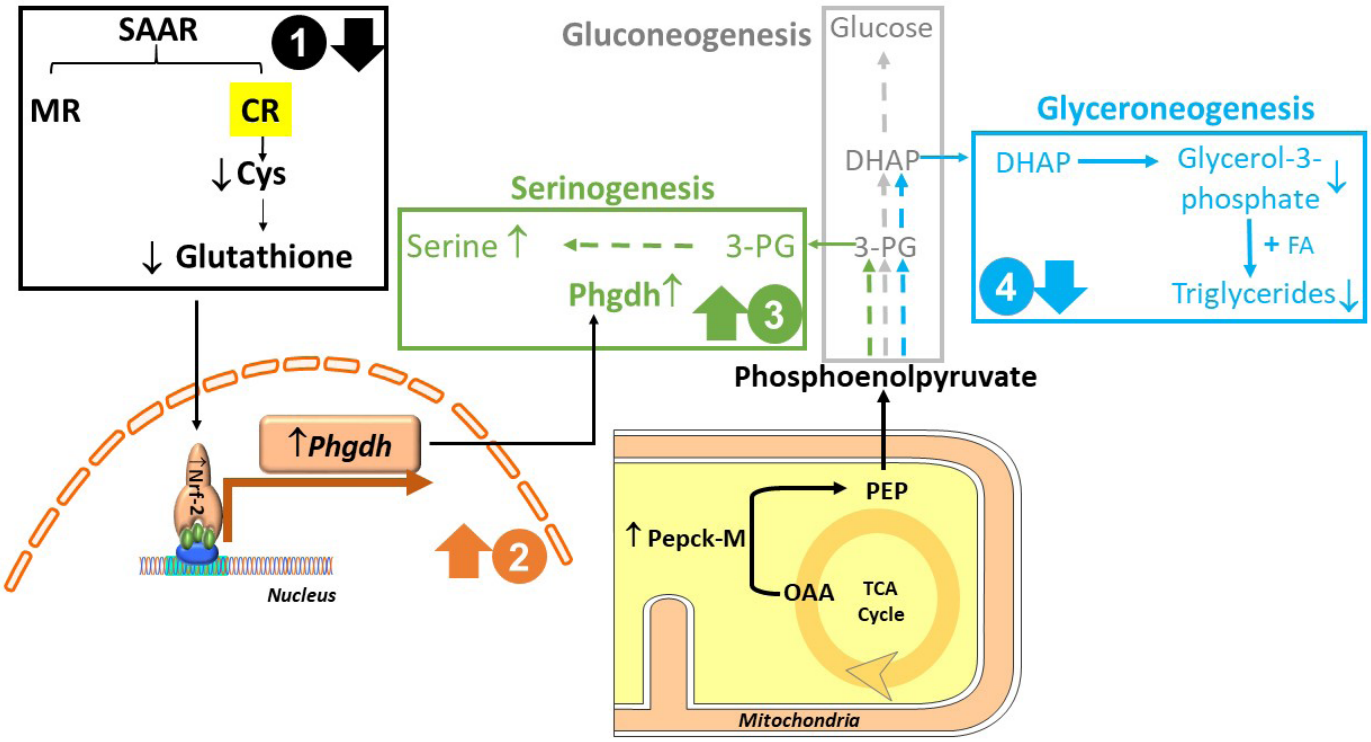
presence of Cys. Accordingly, he aimed to determine whether MetR and CysR exert discrete effects on SAAR-induced benefits—in particular, on lipid metabolism.

By conducting a series of experiments, Dr. Nichenametla found a potential mechanism by which CysR (but not MetR) might be decreasing body fat in animals. Based on the data generated by his team, he proposes that animals on CysR use biochemical intermediates essential for the synthesis of triglycerides (the structural units of fat stored in the adipose depots of our body) to synthesize the amino acid serine. By collaborating with researchers at the University of Oslo and Charles University, he found that data from humans is consistent with the mechanism he found in rodents. In humans, he found that higher plasma serine and lower plasma cysteine are associated with lower levels of plasma triglycerides and a lower incidence of the disease known as metabolic syndrome. Overall, he proposes that increasing serinogenesis (serine synthesis) might divert the biochemical intermediates required for triglyceride (fat) synthesis and eventually cause loss of body fat. His future research will be designed to confirm the mechanism he proposed, and to find ways to increase serinogenesis.

This year, Dr. Nichenametla was awarded the American Society for Nutrition's Vernon Young International Award for Amino Acid Research. He also received a Pilot Award from Nathan Shock Center-San Antonio to study "Circadian Rhythms-Associated Effects of SAAR" and an Academic Grant from Taconic Biosciences to study "Protein Misfolding in the Liver".

Figure 3. The proposed mechanism by which cysteine restriction affects lipid metabolism



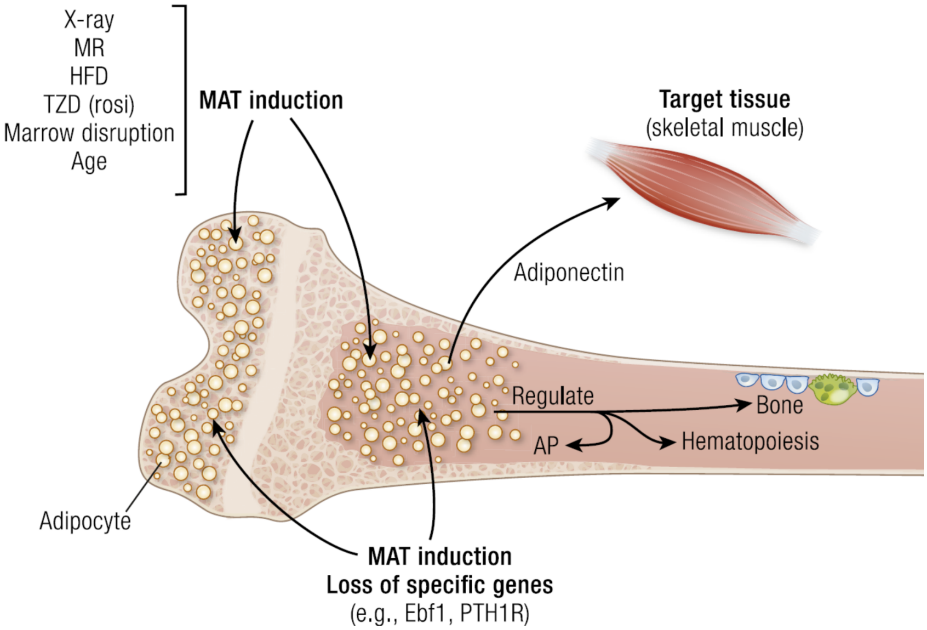
The proposed mechanism of CysR-specific effects of SAAR on adipose metabolism. 1) Due to the lack of Cys in SAAR diets, it results in both MetR and CysR. CysR specifically results in decreased biosynthesis of the tripeptide glutathione. 2) Lower hepatic glutathione increases the abundance of the transcription factor Nrf2, which translocates to the nucleus and induces the transcription of Phgdh. 3) Increase in the transcription and translation of Phgdh and Pck-2 results in higher hepatic serinogenesis (serine biosynthesis from substrates other than glucose, i.e., from oxaloacetic acid). 4) Higher serinogenesis competes with glyceroneogenesis as both pathways use the same set of substrates, which results in lower fatty acid re-esterification. Note: Broken arrows represent the presence of other biochemical intermediates not shown in the figure. Up and down arrows adjacent to the metabolic intermediates represent an increase and decrease, respectively. 3PG: 3-phosphoglycerate, OAA: Oxaloacetic acid, DHAP: Dihydroxyacetone phosphate, PEP: Phosphoenolpyruvate, FA: fatty acids. *Reprinted from Nichenametla et. al., 2022* Aging Cell*, e13739.*

Dr. Ables previously reported that sulfur amino acid restriction (SAAR) in rodents results in smaller body size and bone mass due to reduced expression of genetic markers that are involved in bone formation (Ables, et al., 2012, *PLoS One* 7, e51357; Ouattara et al., 2016 *Bone Rep* 5, 33). He then verified that SAAR can directly reduce the same bone formation markers by cell culture methods (Ouattara et al., 2016 *Bone Rep* 5, 33).

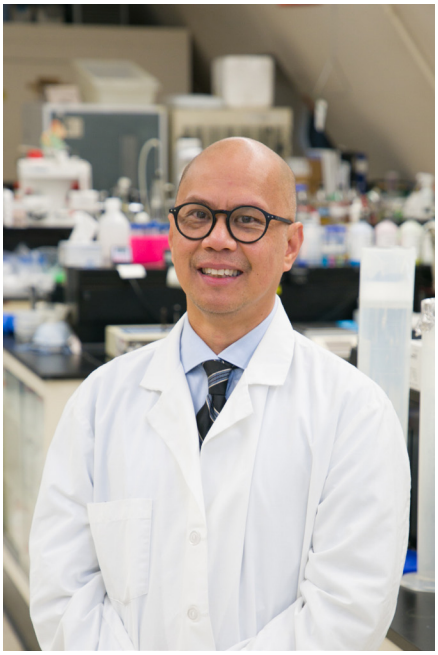
Despite causing this reduction in bone mass, SAAR induces accumulation of bone marrow adipose tissue (BMAT). Marrow fat can also be increased in the long bones of mice by a variety of external inducers, including x-irradiation, rosiglitazone, and a high-fat diet. The mechanisms behind this remain unclear. Functionally, BMAT has been shown to be a source of adiponectin, a glucose sensitizing hormone. It is suggested that BMAT accumulation could be due to increased adipogenesis and decreased lipolysis. Thus, Dr. Ables seeks to identify the mechanism by which SAAR promotes BMAT accumulation. Intriguingly, SAAR results in accumulation of BMAT while peripheral adipose tissue depots, such as perigonadal and subcutaneous, are reduced compared to animals on standard diets.

The Ables laboratory also engages in a variety of collaborations with research teams across the country. At present, they are working on a pilot study with Drs. Mark Horowitz and Doug Rothman of Yale University to determine if high-resolution MRI is suitable for measuring adipocyte depots in mice. His team is also collaborating with Drs. Calvin Vary, Robert Koza, Rea Anunciado-Koza, and Lucy Liaw of MaineHealth Institute for Research to examine the roles of different adipose tissue depots that are affected by SAAR. Additionally, Dr. Ables is engaged in studies involving SAAR with Dr. Andrey Parkhitko of the University of Pittsburgh and with Dr. Tom Hampton of Mouse Specifics, Inc. He is also currently working with data analyst Manuel Marcaida III of Cornell University to analyze RNASeq, proteomics, and lipidomics data from SAAR samples.

Figure 4. Marrow adipogenesis



Marrow adipogenesis can be increased in long bones of C57Bl/6J mice by a variety of external inducers, including x-irradiation (X-ray), physically disrupting the bone marrow, or feeding a specialized diet [containing rosiglitazone (rosi), methionine-restricted (MR), or high-fat (HFD). Alternatively, loss of certain genes (e.g., Ebf1, Pth1r) can also result in increased marrow adipogenesis. Secretion of adipokines, such as adiponectin, can regulate cells outside the bone marrow in an endocrine manner. AP, adipocyte progenitor; TZD, thiazolidinedione. *Reprinted from Sebo et al., 2019* Endocr Rev *40, 1187.*



Gene Ables, Ph.D.
Associate Research Scientist

Dong Laboratory

Dr. Dong's research investigates the translational implications of low sulfur amino acid (SAA) diets on chronic disease prevention in humans.

Epidemiological studies show that diets high in protein are associated with elevated risk of type 2 diabetes (T2D); however, this association depends on protein quantity and source. While diets high in protein from red meat show a positive association with T2D, higher intake of proteins from legumes and seafood is inversely associated with T2D risk, even after adjusting for total energy intake.

In general, plant proteins have a lower concentration of the two proteinogenic SAAs, methionine (Met) and cysteine (Cys). For example, SAA levels in legumes, which are considered high in SAA among plant protein sources, contain only around 25% of the SAAs found in most animal-derived foods. This value drops to about 10% for most other plant protein sources. The Recommended Daily Allowance for total SAAs among adults 19 years and older is 19 mg/kg/day. Data from the National Health and Nutrition Examination Survey show that, in general, Americans far exceed this recommendation.

In previous epidemiological studies, dietary protein intake was assessed only at a single point in time, and no data were available on the impact of long-term SAA consumption on incident risk for diabetes. Therefore, Dr. Dong is investigating habitual dietary SAA intake and long-term risk for diabetes in two ongoing prospective cohorts of the Framingham Heart Study (FHS). Her recently published results indicate that higher cumulative SAA consumption leads to higher risk of T2D development in humans (Dong, et al., 2022 *J Nutr*, 152; 2419). These associations are independent of traditional diabetes risk factors, including cardiovascular disease (CVD) history. Together with previous epidemiological studies, these findings provide insights into the importance of dietary SAAs as a modifier of T2D risk and the potential for reduced SAA intake as a novel dietary intervention to prevent T2D.

Zhen Dong, DrPH
Affiliated Assistant Scientist

Dr. Dong is subsequently looking into links between SAA intake and other chronic diseases such as CVD. Despite T2D and CVD being commonly associated with similar metabolic biomarkers, and the previous results linking SAA intake with a variety of CVD risk factors, direct examination of associations between dietary SAA intake and CVD in humans have yielded inconsistent results. Preliminary results from Dr. Dong's current work, again using the FHS cohorts, indicate that cumulative consumption of higher Met, but not Cys, lead to higher risks of CVD mortality. Detailed mechanisms and explanations are yet to be discovered and will be examined in future work.

The findings of a positive relationship between SAA intake and T2D risk are of significant public health importance given the high rates of T2D and high intake of SAAs in many developed countries. Together with previous preclinical data, there is strong evidence for a novel dietary approach for chronic disease prevention based on the reduction of SAA intake.

Norman Orentreich Award for Young Investigator on Aging

OFAS presented the Dr. Norman Orentreich Award for Young Investigator on Aging to two recipients this year. In May, Dr. Cara Green (University of Wisconsin, Madison) received the award at the American Aging Association 50th Annual Meeting (San Antonio, Tex.). Maximilian Schmid-Siegel, a Ph.D. candidate at the Medical University of Vienna Institute of Medical Genetics (Vienna, Austria) was given the award in July at the 15th International Symposium on Neurobiology and Neuroendocrinology of Aging (Bregenz, Austria). 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, the recipients are invited to present at a future OFAS Symposium.

Publications & Presentations

Plummer JD, Johnson JE. Intermittent methionine restriction reduces IGF-1 levels and produces similar healthspan benefits to continuous methionine restriction. *Aging Cell*, 2022; 21: e13629.

Dong Z, Richie JP, Gao X, Al-Shaar L, Nichenametla SN, Shen B, Orentreich D. Cumulative consumption of sulfur amino acids and risk of diabetes: a prospective cohort study. *Journal of Nutrition*, 2022; 152(11): 2419.

Nichenametla SN, Mattocks DAL, Cooke D, Midya V, Malloy VL, Mansilla W, Øvrebø B, Turner C, Bastani NE, Sokolová J, Pavlíková M, Richie JP, Shoveller AK, Refsum H, Olsen T, Vinknes KJ, Kožich V, Ables GP. Cysteine restriction-specific effects of sulfur amino acid restriction on lipid metabolism. *Aging Cell*, 2022; e13739.

Liaw L, et al. Effects of dietary methionine restriction on age-related changes in perivascular and beiging adipose tissues in the mouse. *Obesity*, in press.

Cooke D, Ruseskas J, Izquierdo B, Hampton T, Ables GP. The impact of dietary sulfur amino acid restriction on amyotrophic lateral sclerosis using B6.SOD1 G93A mice (poster). Neurodegeneration and Proteostasis, New York Academy of Sciences, New York, N.Y.

Johnson JE. Supplementation with amino acids that inhibit insulin/IGF-1 signaling, protect against diet-induced obesity, and improve healthspan (invited talk). University of Virginia Department of Biochemistry and Molecular Genetics Seminar Series.

Johnson JE. Intermittent methionine restriction reduces IGF-1 levels and produces similar healthspan benefits to continuous methionine restriction (oral presentation). American Aging Association 50th Annual Meeting, San Antonio, Tex.

Nichenametla SN. Cysteine restriction-specific effects of sulfur amino acid restriction (SAAR) on lipid metabolism (invited talk). University of Hyderabad Department of Biotechnology & Bioinformatics.

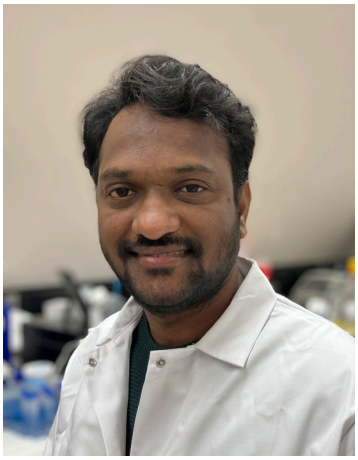
Nichenametla SN. Sulfur amino acid restriction (SAAR) and the mechanism by which it affects lipid metabolism (invited talk). National Institute of Nutrition, Indian Council of Medical Research, Hyderabad.

Ables GP. The effects of dietary sulfur amino acid restriction in mice with amyotrophic lateral sclerosis (oral presentation). 2022 Annual Philippine-American Academy of Science and Engineering Meeting & Symposium, online.

Johnson JE. Supplementation with amino acids that inhibit insulin/IGF-1 signaling, protect against diet-induced obesity, and improve healthspan (invited talk). Albert Einstein College of Medicine Nathan Shock Center Seminar Series.

New Staff

Naidu Babu Ommi joined OFAS as a Postdoctoral Associate in the Nichenametla lab. Dr. Ommi received his Ph.D. in Biotechnology from the Department of Biotechnology and Bioinformatics of the University of Hyderabad (Hyderabad, India). His doctoral thesis examined antiplasmodial activity of fatty acids and macrophage immune modulation during robust erythrophagocytosis. This research originated in his work on cerebral malaria, a severe neurological complication of *Plasmodium falciparum* infection. He also collaborated on studies to screen natural and synthetic compounds, determining their antiparasitic effects against *P. falciparum*.



Dr. Ommi was most recently a Senior Research Fellow at the University of Hyderabad; prior to that he was a Junior Research Fellow at the Rajiv Gandhi Centre for Biotechnology (Thiruvananthapuram, Kerala, India).

Information for Donors

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