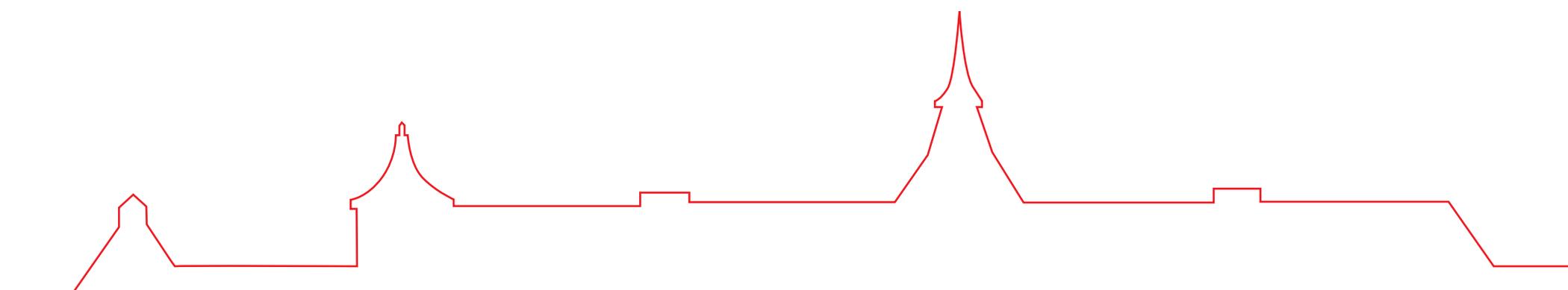




Orentreich Foundation for the Advancement of Science, Inc.





2013 was a banner year for OFAS. In September, we hosted our first scientific conference, “First Annual Mini-Symposium on Methionine Restriction and Lifespan,” in honor of our landmark 1993 paper demonstrating that methionine restriction (MR) led to increased lifespan in rats. As the historical leaders in this specialized field, we provided a unique forum for selected researchers interested in dietary restriction, specifically of the essential amino acid methionine. Attendees greeted this focused convening with enthusiasm, noting that they typically only meet in an ad hoc fashion at major conferences on generalized topics such as obesity.

As we begin the new year, we look forward with excitement to all that 2014 will bring. Our clinical trial with Penn State University examining the metabolic effects of MR in humans will be fully underway. The two senior scientists that joined our staff in December will begin new studies to expand our research on both cancer and MR. And, we will once again co-sponsor and participate in the International Symposium on Neurobiology and Neuroendocrinology of Aging in Bregenz, Austria, continuing our tradition of supporting research in the field of aging.

We persist in our research goal: to develop interventions that prevent, halt, or reverse disorders that decrease the quality or length of life.

Norman Orentreich, MD, FACP

David S. Orentreich, MD

1st Annual Mini-Symposium on Methionine Restriction and Lifespan

With this Symposium, we sought to bring together researchers with an interest in methionine restriction and lifespan, to exchange knowledge, to generate ideas for future investigations, and to strengthen relationships within this community. All these goals were met as attendees enjoyed the opportunity to take a break from their routine to formulate new studies and approaches while gaining access to state-of-the-art, unpublished results. The gathering was the first to exclusively target the focused topic of nutritional restriction. The small size provided a forum in which researchers could directly engage with each other to discuss projects and protocols as well as potential collaborations. The exchange served to give direction to the whole field of research. Attendees addressed methionine metabolism issues from the molecular to the phylogenetic level. Overall, the presentations highlighted the potential importance of MR as an anti-aging strategy and some of the molecular and physiologic pathways that it affects. The Symposium attracted participants from the USA, Europe, Africa, and Asia. It was held on September 9, 2013, at the Tarrytown House Estate & Conference Center in Tarrytown, NY.



David S. Orentreich, MD
OFAS
Welcome Address



Jay A. Zimmerman, PhD
St. John's University/OFAS
Methionine restriction at a glance



George Roth, PhD
GeroScience
Keynote Address
Dietary restriction: Health, longevity, questions, and future direction



Amany Elshorbagy, PhD
Oxford University
Sulfur amino acids and body composition



Holly Brown-Borg, PhD
University of North Dakota
Growth hormone and methionine: Interactions in aging and longevity



Martha Stipanuk, PhD
Cornell University
Regulation of cysteine dioxygenase in response to sulfur amino acid intake: Is minimizing H₂S production the goal?



James Mitchell, PhD
Harvard School of Public Health
Contribution of essential amino acid restriction to the benefits of short-term dietary restriction in mice



Gene Ables, PhD
OFAS
Metabolic effects of dietary methionine restriction in mice



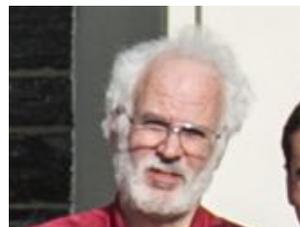
Tsang-hai Huang, PhD
National Cheng Kung University
Effects of methionine-restricted diet and endurance exercise training on bones in male growing rats



Vadim Gladyshev, PhD
Harvard Medical School
Understanding control of lifespan through genome analyses and methionine status



John Richie, PhD
Penn State University/OFAS
Translational studies on dietary methionine restriction



Richard Miller, MD, PhD
University of Michigan
Drugs, diets, genes that extend mouse lifespan: Any common pathways?



Rochelle Buffenstein, PhD
University of Texas Health Science Center
Stress-enhanced proteostasis in long-lived naked mole-rats



Blanka Rogina, PhD
University of Connecticut Health Center
INDY mutations maintain fly health and homeostasis

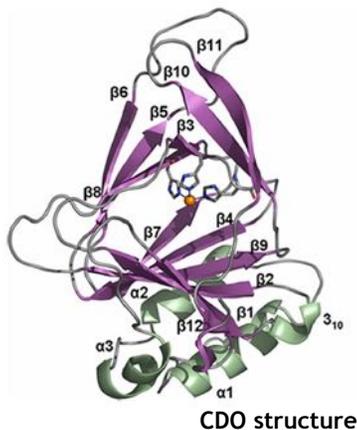


Chris Church, PhD
Harwell Science & Innovation Campus/Yale University
The fat mass and obesity associated gene (FTO) regulates body composition and energy homeostasis

Molecular: What biochemical pathways are changed in MR?

Met/Cys pathway: While methionine (Met) is the only sulfur-containing essential amino acid, mice with mutations inhibiting the synthesis of cysteine (Cys), a sulfur-containing amino acid that can be formed from Met, show the same lean body type and decreased activity of the lipogenic enzyme SCD1 as seen in MR. Dr. Amani Elshorbagy presented data from an OFAS/Oxford University collaborative study showing that addition of cysteine to the MR diet induced hepatic SCD1 and increased fat mass. Dr. John Richie's lab at Penn State University presented recent preliminary results showing that a short term MR (but not Cys restricted) diet lowered plasma Met, Cys, total cholesterol, LDL, glucose, and uric acid in healthy human adults, but had no effects on leptin, adiponectin, IGF-1, or glutathione. Because Cys can be introduced as a Met metabolite or as a food component, the full range of benefits seen in MR appears to require reduced Cys levels in the diet as well. OFAS will optimize the amounts of Met and Cys in a human study in collaboration with Penn State (*see Clinical Trial*).

Dr. Martha Stipanuk of Cornell University presented work on mice lacking Cys dioxygenase (CDO), an enzyme that catabolizes Cys. CDO is found in tissues such as liver, pancreas, fat, and kidney and is itself regulated by how much Cys is available, either ingested or metabolized from Met. While CDO mutations are associated with connective tissue disorders, these mice are lean and resistant to obesity. *Cdo* knockout mice have reduced hypotaurine/taurine and metabolize Cys by desulfhydration, which results in excess levels of sulfide (H_2S , or HS^-). The latter is toxic and can adversely affect the mitochondrial electron transport chain. The *Cdo* knockout mouse serves as valuable tool to study cysteine metabolism; the significance of elevated H_2S (whether as a beneficial signaling molecule or a toxin), as well as lack of hypotaurine/taurine, are the subjects of future investigations.

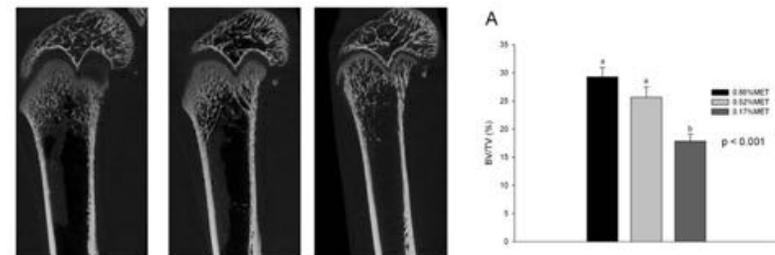


GH/Met interactions: Reduced signaling of the growth hormone (GH) pathway is, like MR, associated with extended lifespan in several species. Ames mice, which have a mutation causing them to lack GH-producing cells in the pituitary, and GH receptor/GH-binding protein knockout mice are—like animals on an MR diet—smaller and leaner and live longer than wild-type mice. Dr. Holly Brown-Borg of the University of North Dakota, in a study still ongoing, has found that MR enhanced median survival of GH transgenic mice, but not the already long-lived Ames mice, who constitutively have elevated levels of antioxidant enzymes and decreased IGF.

What are the tissue-level physiological effects of MR?

Vascular: Dr. James Mitchell's lab at Harvard University is studying the hypothesis that dietary restrictions such as MR are a form of stress to which organisms respond by upregulating endogenous protective factors. Preconditioning mice by short-term dietary restriction, protein restriction, or specific amino acid restriction (such as tryptophan, leucine, or met) has been shown to be protective against surgical stress such as kidney and liver ischemia reperfusion injury (IRI). IRI is a complication of cardiovascular surgery and a significant cause of mortality and morbidity due to stroke and heart attack. Continued research in this area to determine the time needed to see a beneficial outcome from dietary restriction, as well as the molecular mechanism behind it, has the potential to influence patient care, especially before elective surgery.

Bones: Previous OFAS studies have shown MR mice (even those fed a high-fat diet) were metabolically healthy—showing resistance to the usual fat gain and the glucose intolerance seen in animals fed a high fat without MR—but had a smaller body size and lower bone mass than control-fed (CF) animals. In collaboration with OFAS, Dr. Tsang-hai Huang of the National Cheng Kung University in Taiwan conducted experiments to assess the effect of MR and MR plus exercise (EXE) on bone growth. In both studies, MR reduced longitudinal growth and trabecular (spongy) bone volume. While MR and EXE decreased whole bone strength and bone metabolic indices such as osteocalcin and CTX, the intrinsic bone mechanical properties were not compromised.



BV/TV

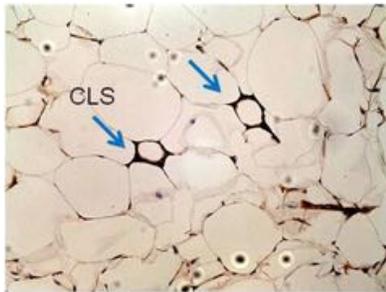
Adipose tissue: Emerging evidence suggests that activation of macrophages by lipid metabolites and subsequent cascading dysregulation are important mechanisms underlying obesity-associated insulin resistance and other side effects of metabolic syndrome. Dr. Gene P. Ables of OFAS presented results in animals on a high-fat MR diet showing that not only were fat deposits smaller, but markers indicating presence of macrophage infiltration in fat depots were decreased compared with high-fat CF animals.

Phylogenic: Is MR a universal pathway for longevity and lean body type, or are there other mechanisms that parallel it?

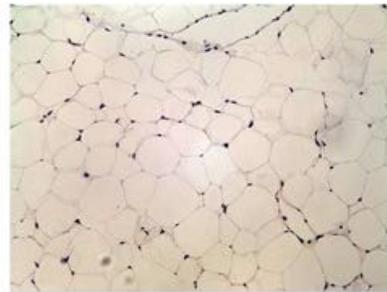
Fruit flies with mutations decreasing expression of Indy, a transmembrane transporter of Krebs cycle intermediates, are—like the Ames dwarf mouse and animals fed a MR diet—smaller and longer lived. Because Indy is linked to transporting Krebs cycle metabolites, it has been posited that mutations in this gene create a calorie-restricted (CR) state. Indeed, CR non-mutant flies have decreased levels of Indy, while mutant flies fed high calorie diets are phenotypically similar to non-mutant CR flies. As discussed by Dr. Blanka Rogina of the University of Connecticut Health Center, the consequences of decreased INDY expression appear to be similar across a diverse range of species, including mammals. Thus, this gene is an attractive target to study age-related metabolic changes and for developing interventions that promote healthy aging.

MR decreased accumulation of macrophages in the adipose tissue following HFD

CF-HFD

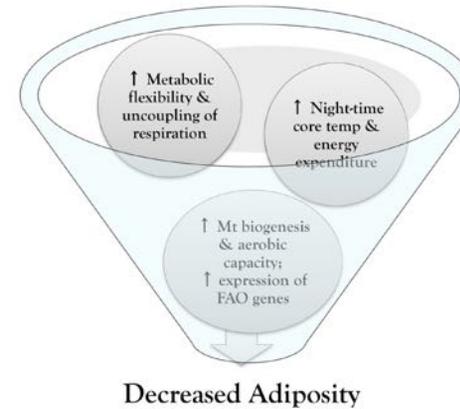


MR-HFD



CLS-crownlike structure

The naked mole rat is the longest-lived rodent, living eight times longer than similarly sized rodents. They remain healthy for most of their life, and their reproductive status is not compromised with age. As herbivores, their diet is low in methionine. Resistance to cellular stress is associated with longevity; naked mole rats are resistant to stress despite high levels of oxidative damage. Dr. Rochelle Buffenstein (Barshop Institute of the University of Texas Health Science Center) presented evidence for increased activity and stability of the proteasome in the naked mole rat. Compared to mice, these animals are more resistant to toxic effects of chemotherapeutic agents and heavy metals. The enhanced health and longevity of the naked mole rat might be linked to increased activity and stability of the proteasome.



Why are MR rats resistant to adiposity?

Richard Miller of the University of Michigan discussed a variety of animal models available to the aging researcher. He also presented some of the results from the National Institute of Aging's Intervention Testing Program. This program evaluates therapies (which are not limited to drugs) that might increase life span or slow age-related diseases in mice. Rapamycin was shown to exert dose-dependent increases in life span and slow the aging process. Acarbose, an anti-diabetes drug, also increased survival in mice, but the effect was more pronounced in males. An important area of interest focuses on the pathways or molecular signatures common to longevity models. One such protein, ATF4, is currently being investigated in calorie and methionine restriction, dwarf mice, and rapamycin- and acarbose-treated mice.

Genome-wide association studies have shown that single nucleotide polymorphisms (SNPs) in the human fat mass and obesity gene (FTO) are strongly associated with obesity and type 2 diabetes. Dr. Chris Church of Harwell Science in Oxfordshire, UK, presented data from transgenic mouse models, highlighting the role of FTO in body composition and metabolism. Mice over-expressing FTO are obese while constitutive knock-out of FTO results in reduction in growth, fat mass, and lean fat mass. Several lines of evidence also point to FTO as a nutrient sensor with its levels regulated by availability of essential amino acids, including methionine.



Ames dwarf mouse

Dietary methionine and cysteine restriction in healthy human adults

In October, OFAS began conducting a Phase I study on methionine (Met) and cysteine (Cys) restriction in humans. We hypothesize that a diet containing reduced levels of both Met and Cys will have similar anti-aging effects in humans as those observed in rodent models. This controlled feeding study will take place at the Penn State University Clinical Research Center (Hershey, PA) under the direction of Dr. John Richie. Healthy adults will be provided diets based on a combination of low protein, low sulfur amino acid foods and essential amino acid supplements (medical foods). Comparisons will

be made between MR alone and a combined Met and Cys restriction (M/CR) as well as between different levels of restriction for MR and M/CR diets. Major outcome variables will include body weight, biomarkers of oxidative stress and plasma lipids, amino acids, insulin, leptin, and adiponectin. Plasma proteomic analyses will also be conducted. We anticipate that results from this study will establish for the first time the efficacy of MR and M/CR diets in human subjects for potential use as an anti-aging and aging-related disease prevention strategy. The study is designed for two years at a cost of \$650,000.



Clinical study facilities at PSU

Publications

Malloy VL, Perrone CE, Mattocks DAL, Ables GP, Caliendo NS, Orentreich DS, Orentreich N.

Methionine restriction prevents the progression of hepatic steatosis in leptin-deficient obese mice.

Metabolism: Clinical and Experimental, 2013; 62(11):1651-1661.

Ables GP, Peffers M, Seymour H, Hampton T, Perodín F, Augie I, Orentreich DS, Orentreich N.

Dietary methionine restriction-induced hyperhomocysteinemia does not alter cardiac function in mice.

Manuscript in preparation.

Sinha R, Cooper TK, Rogers CJ, Sinha I, Turbitt WJ, Calcagnotto A, Perrone CE, Richie JP.

Dietary methionine restriction inhibits prostatic intraepithelial neoplasia in TRAMP mice.

Submitted.

New Staff



Julie R Hens, PhD

After receiving her PhD in Animal Science from the University of Maryland (College Park, MD), Dr Hens spent six years at Yale University studying early embryonic mammary gland development. Following this she was an Assistant Professor at St. Bonaventure University (St. Bonaventure, NY). Her research interests are geared toward understanding mammary gland and lung development. She joins our Cell Biology Laboratory as a Senior Scientist.



Sailendra N Nichenametla, PhD

Dr. Nichenametla received his PhD in Integrative Biosciences from Pennsylvania State University (Hershey, PA) under OFAS consultant Dr. John Richie. He investigated the effect of methionine restriction on several biomarkers such as glutathione, 8-hydroxydeoxy-guanosine, and 8-isoprostane in both rodent models and humans. Dr. Nichenametla comes to us from the Department of Health Sciences, South Dakota State University (Brookings, SD) where he has been a Research Associate. He joins our Animal Sciences Laboratory as a Senior Scientist.



Amadou Ouattara, MS

Mr. Ouattara completed his MS in Chemical Engineering at City College of NY. Having worked most recently with pharmaceutical manufacturer Pfizer, Inc., he joins our Animal Sciences Laboratory as a Research Associate.

International Symposium on Neurobiology and Neuroendocrinology of Aging

This conference, to be held July 27-August 1, 2014, in Bregenz, Austria, is part of a biennial series that started in 1992. The program consists of invited lectures, poster presentations, and informal discussions. OFAS will, as in years past, co-sponsor the 2014 sessions.

Symposium 2015

Given the success of our Mini-Symposium in 2013 and the enthusiastic feedback from participants, we will again host a symposium in 2015. The scope will increase to cover more aspects of nutritional restriction, and we hope to increase attendance to bring even more ideas and avenues of research to the mix.





Orentreich Foundation for the Advancement of Science, Inc.



Information for Donors

The Orentreich Foundation for the Advancement of Science, Inc., is a non-profit institution dedicated to biomedical research to prevent, halt, or reverse those disorders that decrease the quality or length of life. A 501(c)(3) non-profit corporation (EIN 13-6154215), OFAS is duly registered with the United States Internal Revenue Service as an Operating Private Foundation under Section 4942(j)(3).

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