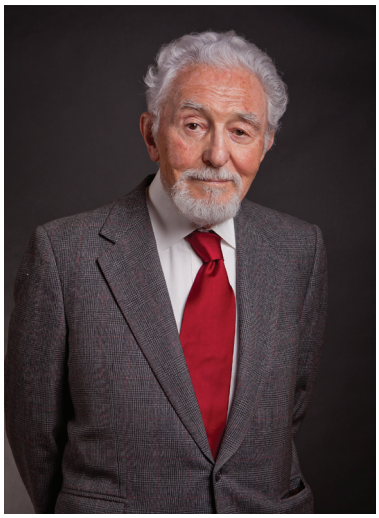
OFAS

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



2014 was a year of growth for OFAS. After two senior scientists joined our staff in December 2013, three senior research associates were added during the past year. Their presence has enabled us to expand the depth and breadth of research we can accomplish.

In addition to three publications, we presented posters at four meetings including a Keystone Symposium in British Columbia and two at Cold Spring Harbor Laboratory, Long Island. The studies we shared were diverse: obesity and insulin resistance, breast cancer cell migration, cardiac function, and lipid/glucose metabolism. Unifying all of these is our desire to identify and understand how dietary restriction of methionine, a sulfur amino acid, decreases the incidence of disease while increasing lifespan.

Our clinical trial examining the metabolic effects of dietary restriction of two sulfur amino acids, methionine and cysteine, in humans is underway at Penn State University. With this study we seek both to confirm that the metabolic changes we have observed with improved animal healthspan translate to humans and also to refine the role of cysteine in these effects.

In September 2015, we will host our second international scientific conference, "Diet, Sulfur Amino Acids, and Healthspan". Following on the success of the 2013 inaugural symposium, we are broadening our scope and expanding the program to bring together more of the leaders in dietary and aging research.

As always, we pursue our goal of developing interventions that prevent, halt, or reverse disorders that decrease the quality or length of life.

Norman Orentreich, MD, FACP
Founder and Co-Director

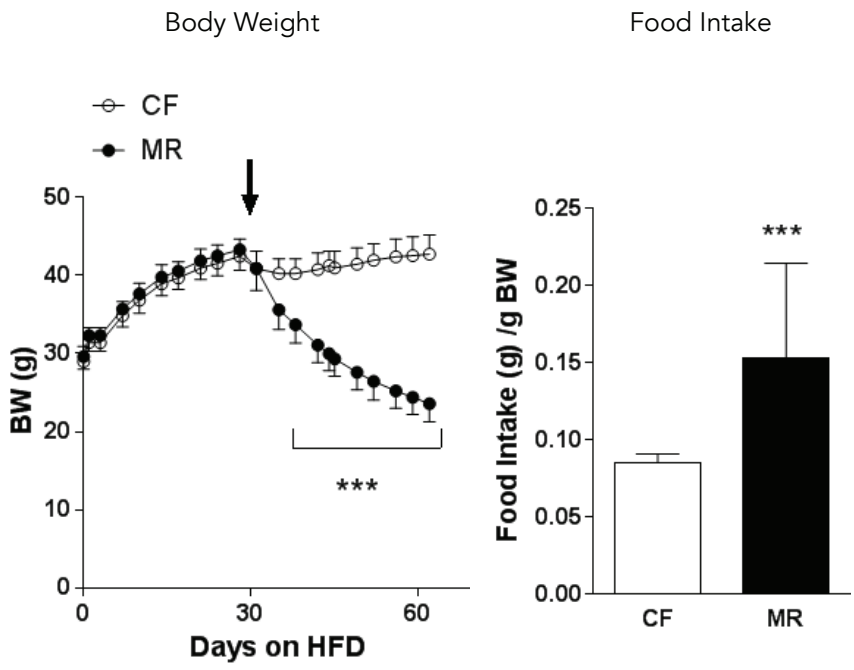
David S. Orentreich, MD
Co-Director

Dietary methionine restriction induces weight loss and reverses insulin resistance in Agouti Yellow (*A^{y}/a*) mice due to increased fibroblast growth factor 21 (FGF21) activity

Ables GP, Peffers M, Seymour H, Ouattara A, Orentreich DS, and Orentreich N

From a poster presented at Complications of Diabetes, Keystone Symposium on Molecular and Cellular Biology, March 23-28, Whistler, BC, Canada

The methionine restricted (MR) diet in rodents extends lifespan and induces favorable metabolic changes on glucose metabolism with a concomitant reduction in adipose tissue mass. The Agouti yellow (*A^{y}/a*) mouse strain is genetically predisposed to develop obesity and diabetes at maturity. Since MR protects mice from diet-induced obesity and diabetes, we hypothesized that it will attenuate metabolic dysfunction in the genetically diabetic *A^{y}/a* mice.



HFD-*Ay/a* mice on the MR diet exhibited weight loss despite having increased food intake.

Mature *A^{y}/a* mice with impaired fasting glucose levels were divided randomly into 2 groups (n = 7-8 per group) and given the control-fed (CF, 0.86% methionine) or the isocaloric MR (0.12% methionine) diet. The MR mice had decreased body weights compared to the CF mice while food intake was similar in both groups. The MR mice had lower levels of fasting glucose, insulin, and leptin while adiponectin and FGF21 levels were higher. Interestingly, MR reversed the insulin resistance in the *A^{y}/a* mice as determined by glucose and insulin tolerance tests. The MR mice had smaller livers and less adipose tissue masses compared to the CF mice upon termination. The MR diet induced hepatic upregulation of *Cd36* and *Fgf21* and downregulation of *Scd1*. A second experiment on high-fat diet (HFD) fed Ay/a mice on MR also showed weight loss, had lower fasting glucose, insulin, and triglycerides with higher FGF21 levels. MR also upregulated hepatic *Cd36* and *Fgf21* and downregulated *Scd1* and *Pparg* expression in *A^{y}/a* mice on high-fat diet. Both studies on a genetic model of diabetes demonstrate that MR increased FGF21 activity, which consequently improved the overall metabolic state.

Conclusion: Taken together, our data suggest that dietary MR in the *A^{y}/a* mice could induce weight loss and reverse insulin resistance due to increased FGF21 activity.

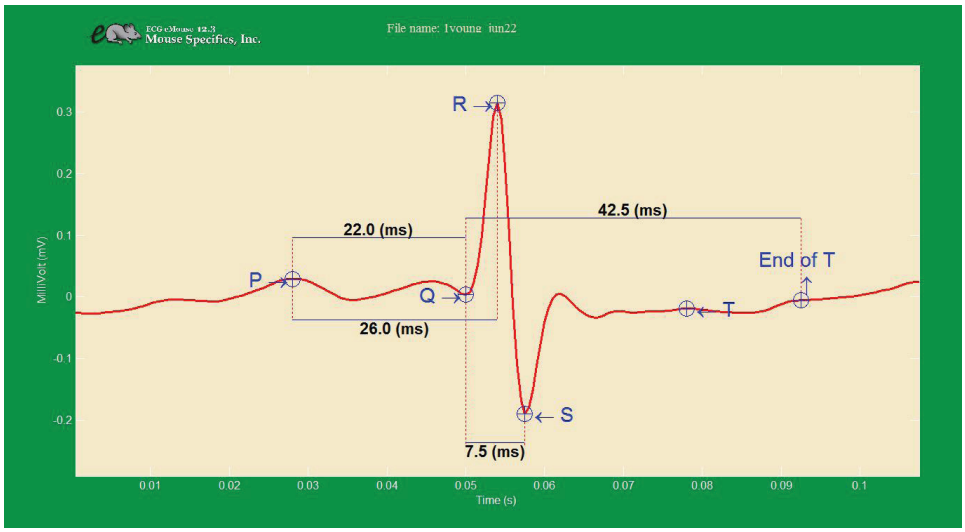
The effect of dietary methionine restriction-induced hyperhomocysteinemia on cardiac function in mice

Ables GP, Peffers M, Seymour H, Hampton T, Perodin F, Augie I, Orentreich DS, and Orentreich N

From a poster presented at Rejuvenation Biotechnology 2014, SENS Research Foundation Conference, August 21-23, Santa Clara, CA

Dietary methionine restriction (MR) extends lifespan in rodents with concomitant hyperhomocysteinemia (HHcy), a condition associated with increased risk for cardiovascular disease. The paradoxical effect of homocysteine on lifespan was, therefore, assessed by testing the cardiac function in young and old mice on the MR diet.

Young (8-20 weeks, n = 7-8/group) and old (60-74 weeks, n = 7-8/group) C57BL/6J male mice were fed a diet containing either 0.84% methionine (control-fed; CF) or 0.12% methionine (methionine-restricted; MR) for 12-14 weeks. We show that young and old MR mice had relative cardiac enlargement and HHcy compared to respective CF groups. In addition, plasma levels of the sulfur amino acids methionine, taurine, and cysteine were lower in the MR mice. Cardiac gene expression analysis revealed upregulation of the hypertrophy markers for natriuretic peptide A (*Nppa*) in young MR mice while *Nppb* was upregulated in both young and old MR mice as compared to CF. Histological analysis showed that cardiomyocyte sizes were similar in both groups of young mice but were smaller in old MR mice than in old CF mice. Immunohistochemistry analysis for cell proliferation marker Ki67 was similar among all four cohorts. Non-invasive electrocardiography (ECG) showed that, at baseline, young MR mice had longer QRS segments than CF mice. However, following an acute response to cardiac hypertrophy by β-adrenergic stimulations using isoproterenol, the response was similar in both groups of young mice. In the old mice, we found no differences between the 2 groups at baseline in all ECG parameters. Interestingly, while old CF mice responded to the isoproterenol injections with longer RR, PQ, and PR segments compared to their own baseline, MR mice did not elicit any response following the stimulation. To test for cardiac contractility, isolated heart retrograde perfusion tests were conducted and revealed similar levels of response at baseline and even after calcium and isoproterenol stimulations in both groups of old mice.



Non-invasive ECG.

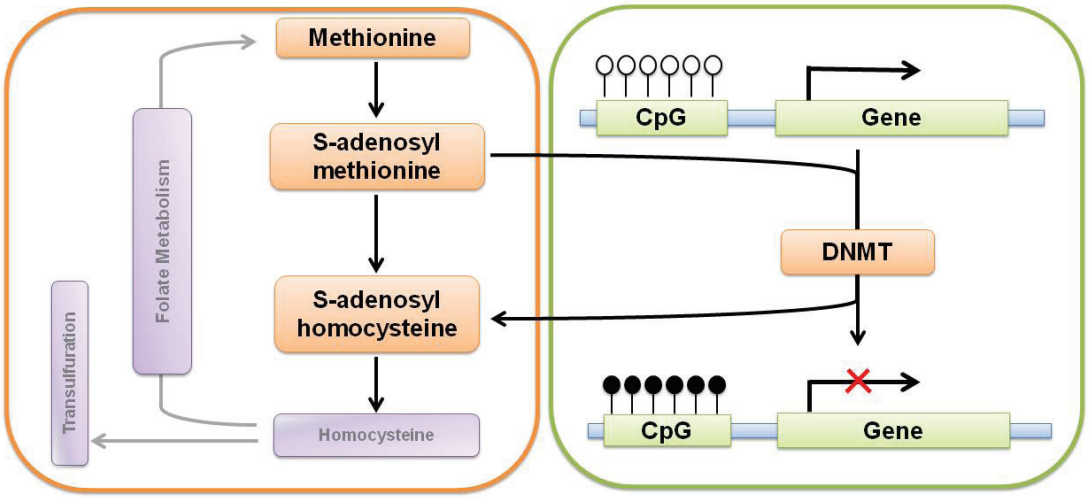
Conclusion: Overall, our studies suggest that MR-induced HHcy may not affect cardiac function in mice.

Role of DNA methylation in transcriptional changes induced by methionine-restricted diets

Nichenametla SN, Mattocks D, Shneyder J, Ables GP, Liu X, Mentch S, Locasale J, Orentreich DS, and Orentreich N

From a poster presented at Epigenetics & Chromatin, Cold Spring Harbor Laboratory, September 9-13, 2014, Cold Spring Harbor, NY

Low methionine diets (MR) are known to extend lifespan and ameliorate diabetes and obesity in animal models, but little is known about molecular mechanisms. Recently, MR-induced changes in glucose and lipid metabolism were associated with a modified transcriptional profile. Some studies also suggest such modifications might be affected by age. Since DNA methylation is susceptible to dietary methionine levels and aging, we hypothesized that altered DNA methylation contributes to MR-induced modifications in transcriptional profile.



Dietary methionine is a precursor for SAM, the only substrate for the DNMT catalyzed maintenance of DNA methylation. Altered DNA methylation is a potential mechanism for MR-induced changes in the transcriptional profile of genes involved in glucose and lipid metabolism.

Compared to CF, MR resulted in improved glucose and lipid parameters in both young and adult mice. Fasting glucose levels in adult mice might be more responsive to MR. The effect of MR on S-adenosylhomocysteine (SAH) was tissue-specific. MR decreased SAH levels in liver, increased in adipose tissue, and had no effect in skeletal muscle. Except for a decrease in adult liver, MR did not change S-adenosylmethionine/SAH ratio. The effect of MR on DNA methyl transferase-1 (DNMT1) was also tissue-specific and reflected changes in SAH, an inhibitor of DNMT1. MR increased DNMT1 in adult liver (decreased SAH), decreased in adult adipose tissue (increased SAH), and had no effect in skeletal muscle. Despite differences in SAH and DNMT1, no changes were observed in percent methylation of long interspersed nuclear elements-1, an index of global DNA methylation. We are currently investigating whether MR affects the gene-specific DNA methylation of those genes involved in glucose and lipid metabolism.

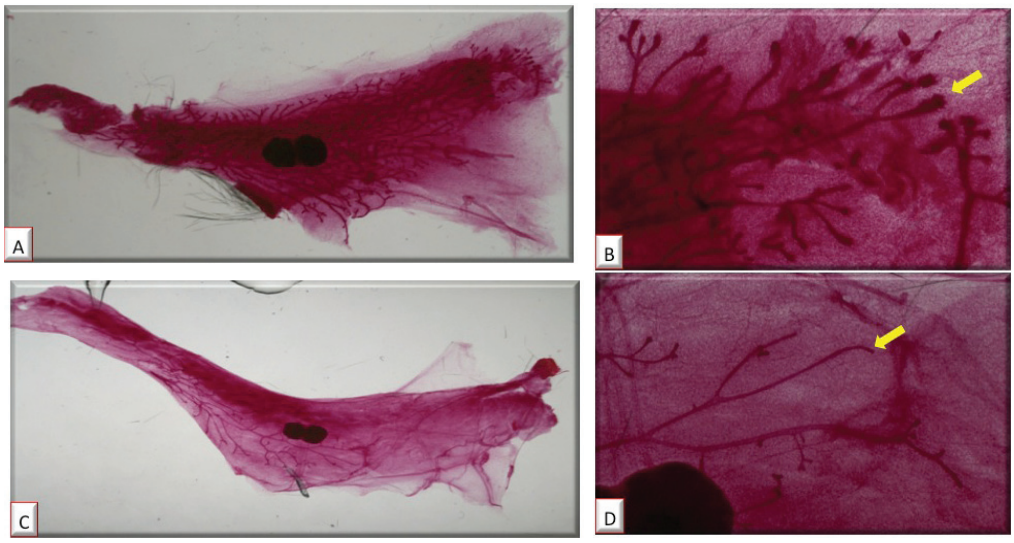
Conclusion: Changes in glucose and lipid metabolism induced in MR do not appear to be due to changes in global DNA methylation. It remains to be investigated whether the improved phenotype is due to changes in gene-specific methylation. Results suggest that the effects of MR on glucose and lipid metabolism could be tissue- and age-specific.

Dietary restriction of methionine inhibits mechanisms that regulate cell migration in mice and breast cancer cell lines

Hens JR, Plummer JD, Perodin F, Orentreich DS, and Orentreich N

From a poster presented at Molecular Genetics of Aging, Cold Spring Harbor Laboratory, September 29-October 3, 2014, Cold Spring Harbor, NY

Mammary glands (MG) are needed in rodents to support new offspring and are comprised of many different cell types including adipocytes. Dietary methionine restriction (MR) extends lifespan and promotes healthy aging in rodents in part by reducing body weight and fat content in the body. MR was examined in CD1 and C57BL/6J mice to determine the effect on MG development and physiology. Three- and eight-week-old female mice were fed isocaloric, L-cystine free diets containing 0.86% methionine (Met) (CF) or 0.12% Met (MR) for five weeks. Histology of the MG revealed that the mammary ductal tree was arrested in MR mice. Transcription factors Zeb1, Twist, Snail1, Snail2, β-catenin, and Lef1, which regulate cell migration, were measured by qRT-PCR. Expression of Zeb1, β-catenin, and Lef1 were increased in MR, while expression of Snail1 was decreased. Snail2 was not detected, and Twist expression was unchanged. Cadherin-11, which may function in cell migration, was down-regulated in MR mice, suggesting that reduced dietary Met hinders cell migration during MG development and growth, and that this might be mediated by mesenchymal cadherin-11. When cells from breast cancer cell line MBMD231 were grown for 24 h in MR (3.45 mg/L vs. control; 17.24 mg/L Met) DMEM/F12 media, expression of cadherin-11 and LEF1 was decreased, while E-cadherin expression was increased. Scratch tests conducted on MBMD231 and MCF10a cells grown in MR conditions showed impaired ability to migrate into cleared space from the scratch. Collectively, these results suggest that MR alters mechanisms associated with cell migration and may cause a mesenchymal-to-epithelial reversal, thereby limiting the metastatic properties of breast cancer cells. This may be of clinical significance for the treatment of metastatic cancers.



Branching morphogenesis in the MG is inhibited in 3½-week-old B6 mice placed on 0.12% MR diet for 5 weeks as compared to the same age mice on 0.86% control diet. MG of mice on MR diet had decreasing numbers of secondary and tertiary branching, in addition to a loss of terminal end buds (indicated by arrows). A and C magnification = 1x; B and D magnification = 6x on a stereomicroscope.

Conclusion: Dietary MR may be beneficial in people with cancer since reduced Met levels inhibit the mechanism of cell migration and may prevent the cancer from becoming metastatic.

Dietary methionine and cysteine restriction in healthy human adults

We began a Phase I study on methionine (Met) and cysteine (Cys) restriction in humans. We hypothesize that a diet containing reduced levels of one or both of the sulfur amino acids Met and Cys will have similar effects in humans as those observed in rodent models. This controlled feeding study is taking place at the Penn State University Clinical Research Center (Hershey, PA) under the direction of OFAS consultant Dr. John Richie. Healthy adults are provided diets based on a combination of low protein and low sulfur amino acid foods, and essential amino acid supplements (medical foods). Comparisons are being made between MR alone and a combined Met and Cys restriction (MR/CR) as well as between different levels of restriction for MR and MR/CR diets. Major outcome variables include body weight, biomarkers of oxidative stress, and plasma lipids, amino acids, insulin, leptin, and adiponectin. Plasma proteomic analyses are also being conducted. With this study we seek both to confirm that the metabolic changes we have observed with improved animal healthspan translate to humans and also to refine the role of cysteine in these effects.

New Staff

To assist our Senior Scientists with a growing number of studies, we added three Senior Research Associates to our staff this year.

Diana Cooke, MS

Diana received her MS in Forensic Molecular Biology from the University at Albany, State University of New York. Her recent previous experience includes working with the NY City Office of the Chief Medical Examiner, Department of Forensic Biology.

Mimi Davis, MA

Mimi received her MA in Biomolecular Sciences from Central Connecticut State University, New Britain, CT. She most recently worked as a Biomarker Assay Scientist at Pfizer, New Haven, CT, and as a Research Associate for Central Connecticut State University.

Jelena Shneyder, PhD

Jelena earned her PhD in Biochemistry from the Peoples' Friendship University of Russia in Moscow. She previously worked at the Roswell Park Cancer Institute, Buffalo, NY, and at Albany Medical College Center for Cell Biology & Cancer Research.

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.

Prostate 2014; 74(16):1663-73.

Ables GP, Brown-Borg HM, Buffenstein R, Church CD, Elshorbagy AK, Gladyshev VN, Huang TH, Miller RA, Mitchell JR, Richie JP, Rogina B, Stipanuk MH, Orentreich DS, Orentreich N.

The First International Mini-Symposium on Methionine Restriction and Lifespan.

Frontiers in Genetics, 2014; 5:00122.

Huang TH, Lewis JL, Lin HS, Kuo LT, Mao SW, Tai YS, Chang MS, Ables GA, Perrone CE, Yang RS.

A methionine-restricted diet and endurance exercise decrease bone mass and extrinsic strength but increase intrinsic strength in growing male rats.

Journal of Nutrition 2014; 144(5): 621-30.

Symposium 2015: Diet, Sulfur Amino Acids, and Healthspan

In 2013, as part of our commitment to promoting the exchange of knowledge and strengthening relationships in the scientific community, we inaugurated a series of symposia that would focus on issues concerning diet and aging. With these symposia, we seek to bring together researchers with an interest in nutritional restriction and aging, to exchange knowledge, to generate ideas for future investigations, and to strengthen relationships within this community. Attendees enjoy 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. This is the first gathering to exclusively target the focused topic of nutritional restriction. The small size provides a forum in which researchers can directly engage with each other to discuss projects and protocols as well as potential collaborations. The symposia attract international participation.

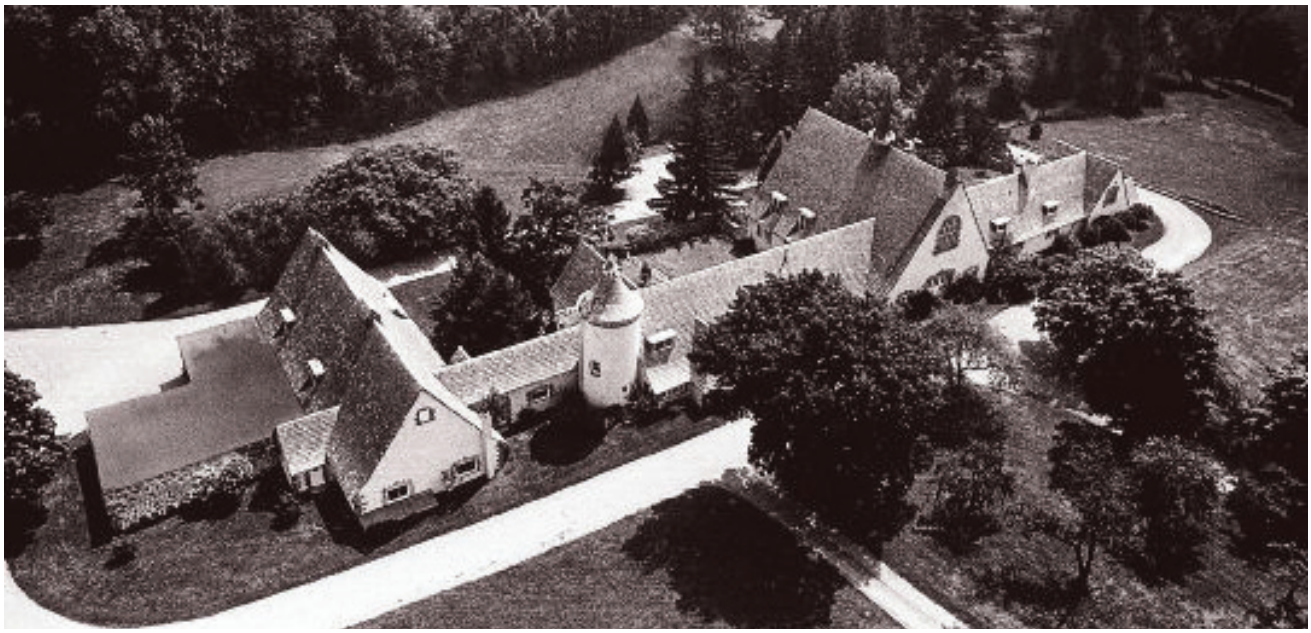
Symposium 2015:
Diet, Sulfur Amino Acids, and Healthspan
September 20-22, 2015, Tarrytown, NY

The keynote address will be given by Dr. Caleb E. Finch (USC Davis School of Gerontology), a leader in the study of aging and inflammation. Full papers from speakers will be published in the international, peer-reviewed journal *Annals of the New York Academy of Sciences.*

OFAS

Orentreich Foundation for the Advancement of Science, Inc.

The Orentreich Foundation for the Advancement of Science, Inc., is dedicated to biomedical research to prevent, halt, or reverse those disorders that decrease the quality or length of life.



Biomedical Research Station in Cold Spring-on-Hudson, NY
Staff enjoy state-of-the-art facilities housed in a 100-year-old building designed by noted architect Louis Colt Albro.

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). No accomplishment of OFAS is possible without your encouragement and generous support.
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