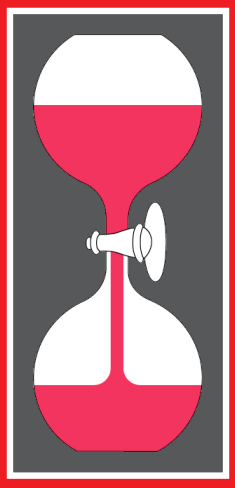
OFAS

REPORT OF THE DIRECTORS

December 2010

Orentreich Foundation for the Advancement of Science, Inc.

OFAS is dedicated to biomedical research that prevents, halts, or reverses those disorders that decrease the quality or length of life.



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

Letter of the Directors

Dear Friends,

The year 2010 was a remarkable one at OFAS:

- our seminal research on methionine restriction (MR) advanced significantly toward elucidating its positive role in the fight against obesity, cancer, and aging;
- we made significant strides in building collaborations with Oxford University, the University of Oslo, Penn State University, and sanofi-aventis that we anticipate will flourish in 2011;
- we published six papers in peer-reviewed journals and presented at two conferences and two poster sessions, more than doubling our 2009 achievements;
- we completed important systems upgrades and purchased new high-performance laboratory equipment;
- our junior and senior staff attended more conferences and training programs than in previous years;
- our revised web site will be active by year's end;
- we developed an energy conservation plan with a goal of saving up to 20% in energy costs upon full implementation;
- we streamlined laboratory practices to achieve significant end-of-year savings;
- and, last but not least, OFAS celebrated 25 years in our unique, scenic research facility in Cold Spring-on-Hudson.

We are proud of all that has been accomplished in 2010 and especially proud of our scientific staff who work tirelessly to develop measures to delay the onset of disease and aging. We are also thankful for the administrative team's hard work in improving operations.

Our research goals for 2011—with a full list of new and extended projects—are set. We are excited and hopeful that the new year will deliver new discoveries, partnerships, and growth through your continued support and faith in our mission.

Respectfully,

Norman Orentreich, MD, FACP
Founder and Co-Director

David S. Orentreich, MD
Co-Director

2010 Research Projects

Since the late 1980s, OFAS has researched dietary methionine restriction (MR) as a means of extending lifespan. Our studies have shown that reducing intake of this essential amino acid can lead to a remarkable increase in maximum age. In addition, animals maintained on this dietary intervention have lower body weight, less fat accretion, improved insulin sensitivity, and reduced incidence of age-related disease, including cancer. Having established the effects of MR, we now seek to understand the mechanisms by which they are accomplished. This section presents MR projects conducted during 2010. The longevity projects remain ongoing to year 2011 and beyond. Findings and/or conclusions from these studies are highlighted in the descriptions that follow.

Clearance of Excess Dietary Methionine

MR is easy to implement in a controlled laboratory environment; however, this diet presents challenges in everyday life, particularly since most of our society consumes meat, fish, or dairy, which have relatively high amounts of methionine. Using our classic MR model, the F344 rat, OFAS has spent several years developing ways to make MR easier to "put into practice". Most recently, we incorporated glycine, a naturally occurring substance, into the normal rat diet to determine if we could produce some of the salient features of classic MR (lower body weight, increased longevity, etc.). **Our observations suggest that supplementing glycine at a higher than normal concentration results in lower body weight and serum triglycerides as well as a trend toward increased lifespan.** Interestingly, the motor coordination of older "supplemented" rats was comparable to that of MR rats. While we continue to monitor longevity in these animals, in 2011 we will employ metabolomics (see inset, page 6) to elucidate biochemical pathways that might be affected by this intervention.

Cysteine Supplementation of MR diets

Stearoyl CoA desaturase-1 (SCD1) is an enzyme that regulates fatty acid and energy metabolism. Activity of this enzyme is increased in animal models presenting with obesity or liver steatosis. F344 rats on MR have decreased hepatic Scd1 gene expression, increased energy expenditure, and less adipose tissue relative to control animals. MR also reduces serum levels of cysteine, a methionine metabolite. In human beings, total plasma cysteine levels—but not methionine levels—correlate with increased body fat, obesity, and the metabolic syndrome. OFAS, in collaboration with Drs. Amany Elshorbagy (Oxford University) and Helga Refsum (University of

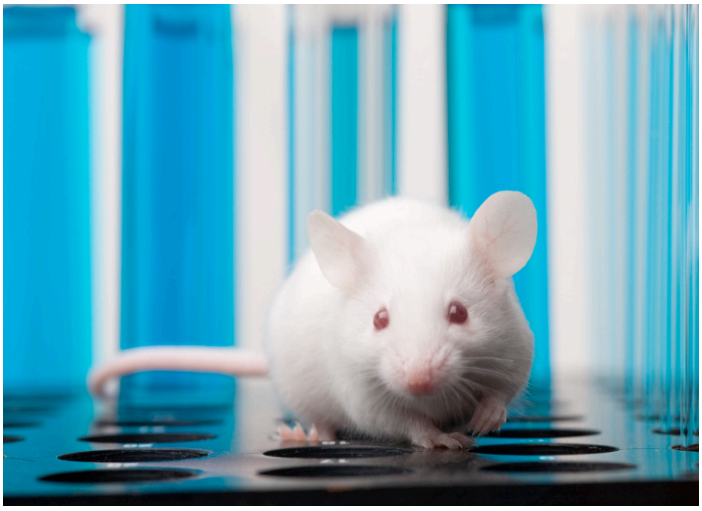
Oslo), conducted studies to determine which metabolic consequences of MR are mediated by reduction in cysteine. At the 28th Annual Scientific Meeting of the Obesity Society, **OFAS presented research showing that the anti-adiposity effects of MR are due to decreased cysteine levels and that adding cysteine to MR diets does not increase methionine levels, since adding cysteine to MR rodents doesn't increase serum methionine levels.** We also presented data showing that SCD1 is suppressed in the liver of MR F344 rats. Conversely, when the MR diet is supplemented with cysteine, the inhibitory effects of MR on SCD1 are reversed. The results identify dietary sulfur amino acid composition as an important regulator of SCD1 function. We have also initiated a large study to determine if the known longevity effects of MR are due to reduced serum methionine or cysteine. Results from these studies will enable us to determine if there are associations between obesity and lifespan.

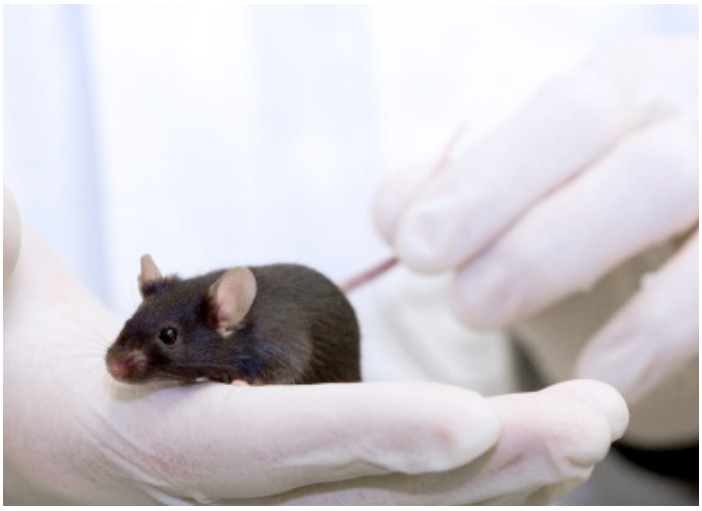
Taurine and Glutathione Supplementation in F344 Rats

Diets used in OFAS research are devoid of all sulfur-containing amino acids except methionine, but it is difficult to assess whether MR's inhibitory effects on adiposity are due to reduced methionine or other sulfur-containing amino acids that originate from methionine's metabolism in the liver. **As we reported in the *Journal of Lipid Research* this year, the adipose inhibitory effects of MR are reversed when cysteine is added to MR diets.** Cysteine is a precursor of two antioxidants, taurine and glutathione, the latter of which is increased in MR rats. MR rats, which have low serum levels of taurine and are lean and insulin sensitive. However, this contradicts studies suggesting that increased taurine consumption is effective in reducing body weight and improving the response to insulin, Studies on MR with taurine supplementation in F344 rats that began this fall will provide evidence for taurine's effects on body weight. A concurrent study on MR with glutathione supplementation will be conducted to determine if MR effects are mediated through changes in glutathione.

MR and Hepatic Steatosis in Obese Mice

The incidence of non-alcoholic fatty liver disease (NAFLD) has risen dramatically due to the obesity/diabetes epidemic. The clinical spectrum of steatosis (infiltration of liver cells with fat) ranges from the simple to more damaging forms, such as non-alcoholic steatohepatitis (NASH) and cirrhosis. Although nearly 20 million Americans are affected by NAFLD, there are no effective





preventions and few treatments. NAFLD is characterized by accumulation of triglycerides in the liver due to excess calorie intake, insulin resistance, and/or dysregulation of fat metabolism. In addition to massive obesity and insulin resistance, obese mice (ob/ob) develop hepatic steatosis early in life. **OFAS has examined the effect of dietary MR on the development of hepatic steatosis in this model and has shown that MR reverses hepatic steatosis in these mice. MR also lowers serum markers of liver damage (*e.g.*, alanine aminotransferase and aspartate aminotransferase) and hepatic triglyceride accumulation.** Reduction of liver damage was confirmed with histologic examination of livers from mice fed the MR diet, which showed no obvious pathology. Also, genes associated with hepatic steatosis were suppressed by MR. We recently presented our studies at the Cold Spring Harbor Molecular Genetics of Aging Meeting and at the 28th Annual Scientific Meeting of the Obesity Society. In 2011, we will use metabolomics (see inset below) to elucidate other beneficial mechanisms associated with MR, which is a potential intervention or treatment for NAFLD.

MR and Chemoprevention in a Rodent Breast Cancer Model

We and others have established that MR, in addition to extending lifespan in rodent models, also reduces spontaneous age-related and chemically-induced diseases. Using the ACI rat, a model that develops breast malignancies similar to those observed in humans, we are exploring the possible chemopreventive effects of MR.

MR and the Mammalian Target of Rapamycin (mTOR) Pathway

mTOR is an intracellular protein reported to be a key regulator of protein syntehsis. Furthermore, abnormalities in the mTOR signaling pathway have been implicated in some forms of cancer. When adequate supplies of nutrients (including amino acids) are available, mTOR is activated, resulting in upregulation of proteins that influence cellular nutrient uptake, proliferation, angiogenesis, and other effects. In contrast, when nutrients are not readily available, mTOR is inactivated and protein synthesis is inhibited. **We examined liver, skeletal muscle, and adipose tissue of MR rats and found that the beneficial effects of MR have so far not shown an effect on mTOR activity in these tissues.** Further investigation is needed.

Heavy Water and Lifespan Extension

We have begun a small study to examine the potential effects of deuterium, a naturally occurring hydrogen isotope, on lifespan extension, specifically on damage caused by free radicals. Mice in this experimental group receive 10% deuterium in their drinking water. As with all our longevity experiments, it will take approximately 18-24 months to report on the results of this study.

What is Metabolomics?

Metabolomics (metabolic profiling) is an emerging research area that involves profiling of small molecule metabolites. These metabolites can be proteins, lipids, or carbohydrates. Tissue or blood samples are processed through sophisticated separation techniques to generate a lab array, which is divided into two sections: known and unknown metabolites. The known metabolites are easily identifiable by comparison against a standard. Some information can also be obtained about unknown metabolites, *e.g.*, structure of the molecule. The molecules are grouped into classes, which enables comparison of levels of metabolites among experimental and control groups. The changes between groups, as well their statistical significance, are also identified. The molecules are then "mapped" to a biochemical pathway to determine how metabolic pathways are impacted by the experimental treatment. Metabolomics is being used in many areas of research including pharmacology and diagnostics.

Collaborations

Oxford University

Because one significant methionine-restriction (MR) effect in rodents is decreased fat mass, current research at OFAS deals with the effects of MR on obesity. In collaboration with Drs. Helga Refsum (University of Oslo) and Amany Elshorbagy (Oxford University), studies were conducted to identify whether methionine or products from methionine metabolism regulate fat mass accumulation. The research was based on results from the Hordaland Homocysteine Study (a population-based study relating homocysteine levels to general health), which revealed a direct correlation between cysteine (a metabolite derived from methionine) and obesity in humans. The supplementation of MR diets with cysteine reversed MR's effects on decreased adiposity in rodents, confirming that cysteine is a key component in fat mass accumulation. Because cysteine supplementation does not restore methionine levels in rats, ongoing research will examine whether the lifespan extension effects of methionine restriction are a consequence of reduced methionine or its metabolite, cysteine. Our continuing research will also provide information to confirm or refute hypotheses postulating that obesity is a key modulator of lifespan.

Penn State University

Dr. John Richie, Professor at the College of Medicine Public Health Sciences Department at Penn State University, joined OFAS as a research consultant. We will be collaborating in the development of protocols to extend MR studies to humans. Dr. Richie is currently directing a human MR study to examine the effects of this dietary intervention on antioxidants such as gluthathione. OFAS plans to work with his team to pursue other MR human studies in the near future.

Kaiser Permanente

In collaboration with Kaiser Permanente Division of Research, we conducted a study to find potential biomarkers that predict heart muscle damage following myocardial infarction (MI). In this study, serum samples from 695 patients with a history of MI and 690 control patients were examined for the presence of factors that induce blood vessel development (angiogenesis), a process involved in the repair of tissues damaged by low oxygenation. The study revealed that biomarkers of angiogenesis, specifically angiopoietin 2, are good predictors of MI.

Serum Treasury

Serum Treasury projects analyze frozen serum samples taken years ago from healthy members of the Kaiser Permanente Medical Care Program of Northern California. Analyses look for factors that predict the risk of development of (or resistance to) a particular disease.

Non-Hodgkin's Lymphoma and Organochlorides, Aflatoxin, and EBV infection

This nested case-control study of non-Hodgkin's Lymphoma was carried out using Serum Treasury samples within a cohort of the Northern California Region Kaiser Permanente Medical Care Program. It analyzed a variety of organochloride levels, EBV seropositivity, and cytokine and immunological biomarkers. At this time OFAS is still 'blind' to the data generated. ***Collaborators:*** *Centers for Disease Control and Prevention, National Institutes of Health, Virolab, Inc, Kaiser Permanente Division of Research, and OFAS*

Breast Cancer and Proteomic Analysis

This study developed a method to reveal the identity and pattern of groups of proteins—and their relative quantity—in persons who did (or did not) go on to develop breast cancer. Further research utilizing the new methodology will need funding. ***Collaborators:*** *Albert Einstein College of Medicine, Kaiser Permanente Division of Research, and OFAS*

Male Breast Cancer

Because the National Cancer Institute (NCI) is starting to look into 'neglected' cancers, OFAS and Kaiser Permanente Division of Research have received NCI contracts to find serum components that change in male breast cancer. Lists of male breast cancer cases and matched controls are being prepared so that OFAS personnel can retrieve the sera from the Serum Treasury and prepare the needed aliquots for testing. ***Collaborators:*** *National Cancer Institute, Kaiser Permanente Division of Research, and OFAS*

Selenium and Thyroid Cancer

This pilot study has been completed. It concerns the durability of selenium in long-frozen sera with an eye to evaluating the suspected association of low selenium with increased cancer risk—in particular, thyroid cancer. A definitive study on any correlation between thyroid cancer and selenium levels in long-frozen, pre-diagnostic sera samples will require funding. ***Collaborators:*** *Kaiser Permanente Division of Research and OFAS*

Liver Cancer and Organochlorides, Aflatoxin, and Hepatitis C Virus

This study examines the association of organochlorides, aflatoxin, and hepatitis C with the subsequent development of liver cancer. It is currently in the measurement phase of serum retrieved from the Serum Treasury, and raw data is expected soon for analysis. ***Collaborators:*** *Kaiser Permanente Division of Research, International Agency for Research on Cancer, Centers for Disease Control, University of Leeds, Gotesborg University, and OFAS*

Liver Cancer, Hormones, and Hepatitis C Virus

This study will examine the relationships between liver cancer, hormones, and the Hepatitis C virus. We are currently preparing lists of patients and matching controls for which Serum Treasury samples will be retrieved. ***Collaborators:*** *Kaiser Permanente Division of Research, National Cancer Institute, Boston Children's Hospital, and OFAS*

Publications & Presentations

Publications

- Perrone CE, Mattocks DAL, Jarvis-Morar M, Plummer JD, Orentreich N. Methionine restriction effects on mitochondrial biogenesis and aerobic capacity in white adipose tissue, liver, and skeletal muscle of F344 rats. *Metabolism*, 2010; 59(7):1000-11.
- Asgari MM, Tang J, Warton EM, Chren M-M, Quesenberry CP, Bikle D, Horst RL, Orentreich N, Vogelman JH, Friedman GD. Association of pre-diagnostic serum vitamin D levels with the development of basal cell carcinoma. *Journal of Investigational Dermatology*, 2010; 130(5):1438-43.
- Plaisance EP, Henagan TM, Echlin H, Boudreau A, Hill KL, Lenard NR, Hasek BE, Orentreich N, Gettys TW. Role of β-adrenergic receptors in the hyperphagic and hypermetabolic responses to dietary methionine restriction. *American Journal of Physiology. Regulatory, Integrative, and Comparative Physiology*, 2010; 299(3):R740-50.
- Hasek BE, Stewart LK, Henagan TM, Boudreau A, Lenard NR, Black C, Shin J, Huypens P, Malloy VL, Plaisance EP, Krajcik RA, Orentreich N, Gettys TW. Dietary methionine restriction enhances metabolic flexibility and increases uncoupled respiration in both fed and fasted states. *American Journal of Physiology. Regulatory, Integrative, and Comparative Physiology,* 2010; 299(3):R728-39.
- Elshorbagy AK, Valdivia-Garcia M, Refsum H, Smith AD, Mattocks DAL, Perrone CE. Sulfur amino acids in methionine-restricted rats: hyperhomocysteinemia. *Nutrition*, 2010; 26(11-12):1201-4.
- Elshorbagy AK, Valdivia-Garcia M, Mattocks DA, Plummer JD, Smith AD, Drevon CA, Refsum H, Perrone CE. Cysteine supplementation reverses methionine restriction effects on adiposity: significance of stearoyl-coenzyme A desaturase. *Journal of Lipid Research*, 2010. *In press.*

Conference Presentations

Presented at Cysteine and Obesity Workshop, Department of Pharmacology, Oxford University, UK, January 26, 2010.

- Perrone Carmen, Methionine Restriction, Lipid Metabolism and Adiposity Resistance (Panel Discussion).

Posters

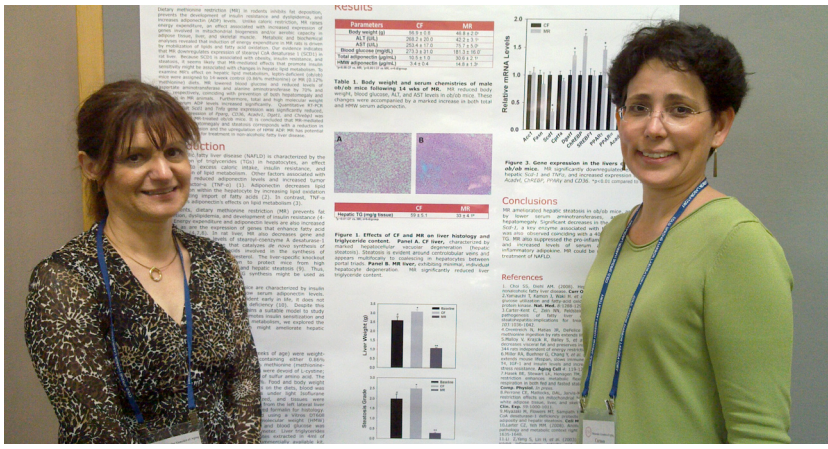
Presented at Obesity 2010, the 28th Annual Scientific Meeting of the Obesity Society, San Diego, CA, October 8-12.

- Elshorbagy AK, Valdivia-Garcia M, Mattocks DAL, Plummer JD, Smith D, Drevon CA, Refsum H, Perrone CE. Cysteine, a Novel Mediator of Adiposity: Regulation of SCD-1 in Rodents.

Presented at Molecular Genetics of Aging, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, September 28-October 2.

- Malloy VL, Perrone CE, Mattocks DAL, Plummer JD, Caliendo NS, Orentreich N. Methionine Restriction Prevents Development of Hepatic Steatosis in Leptin-Deficient (ob/ob) Mice.

Dwight Mattocks, Virginia Malloy, and Jason Plummer attending Molecular Genetics of Aging.



Virginia Malloy and Carmen Perrone attending Obesity 2010.

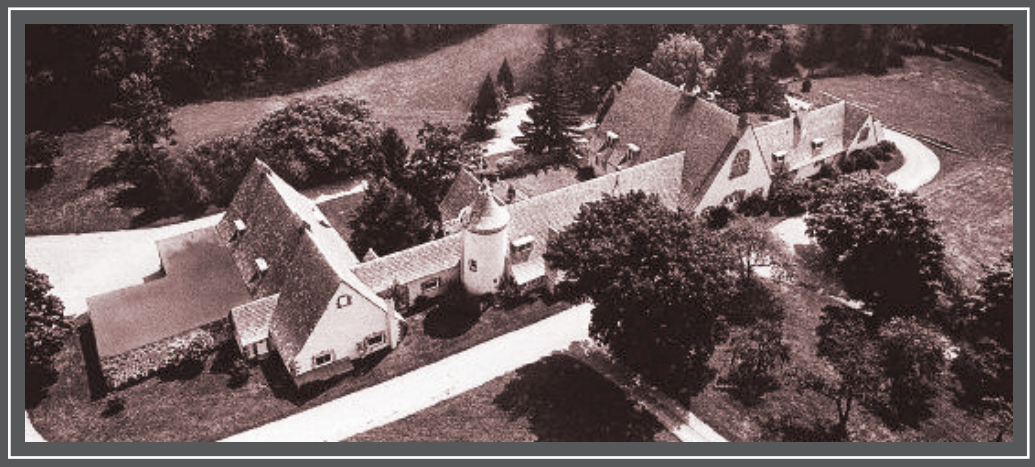
Recent Events

Ana Maria Cuervo
In March we were honored to have Dr. Ana Maria Cuervo, Professor in the Department of Developmental and Molecular Biology at the Marion Bessin Liver Research Center and the Institute for Aging Research at Albert Einstein College of Medicine present a seminar entitled "Autophagy in Aging and Age-Related Diseases." Dr. Cuervo is a leading scientist in the aging field; she introduced the concept of protein degradation through the process of chaperone-mediated autophagy. Her research has provided a better understanding of mechanisms behind neurodegenerative disorders such as Alzheimer's, Huntington's, and Parkinson's diseases.

Summer Student
This year Lovedeep Singh, from the nearby Lakeland High School's Science Research Program, joined OFAS to conduct summer research studies in the Cell Biology Laboratory. Under the supervision of Dr. Carmen Perrone, Mr. Singh examined the expression of the enzyme adipose triglyceride lipase (ATGL) in adipose tissue, liver, and skeletal muscle. ATGL is an enzyme that conducts basal lipid degradation and thus reduces adiposity in rats. His studies revealed that ATGL protein expression levels in adipose tissue, but not in liver, are controlled by cysteine. It was a pleasure to work with Lovedeep and all of us agree that he has a bright future.

Redesigned Website
We are pleased to announce that our website, www.orentreich.org, has been completely redesigned and updated with news of our latest achievements and research. Please visit our new presentation at www.orentreich.org!

Holly Brown-Borg
In November OFAS was privileged to have Prof. Holly Brown-Borg be a key speaker at one of our science meetings. Prof. Brown-Borg is the Chester Fritz Distinguished Professor of Pharmacology and Therapeutics at the University of North Dakota School of Medicine and Health Sciences. She also serves as Chair-Elect of the Biological Sciences Section of the Gerontological Society of America and will be President of the American Aging Association in 2011. She was on the 2010 Organizing Committee of the International Symposium on Neurobiology and Neuroendocrinology of Aging and will Chair the Symposium in 2012. She is best known for her work in elucidating the mechanisms through which the dwarf mouse attains its remarkable longevity.



Information for Donors

The Orentreich Foundation for the Advancement of Science, Inc. was founded in 1961. OFAS 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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