



OFAS

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

Report of the Directors

Dear Friends, Supporters, and Fellow Scientists:

It is hard to believe this is our 48th anniversary and our 22nd year at the Biomedical Research Station in Cold Spring-on-Hudson. The consolidation of our research laboratory facilities, once spread around New York City, into a unified entity has proven remarkably productive. Moreover, the outstanding contributions of our scientists and support staff coupled with our great location make OFAS a uniquely small but successful center for research.

Our Deputy Director, Dr. Bernardita Calinao, continues her day-to-day leadership, overseeing development of research projects, budgets, and program plans with the science directors to ensure efficiency and productivity.

This year, the appointment of Dr. Carmen Perrone as Assistant Director for Scientific Affairs has brought great inspiration to OFAS. Dr. Perrone's 2009 in-house lecture series and publications in major scientific journals have bolstered our resolve to pursue our innovative research in methionine restriction and cancer prevention.

Under the leadership of Senior Scientist Dr. Joseph H. Vogelmann, we continue using the Serum Treasury in successful collaborations with Kaiser Permanente Division of Research. This year five outstanding studies were developed using the Serum Treasury.

It is refreshing to see the three young scientists who joined us in the past year transform into passionate researchers in the fields of methionine restriction, cancer prevention, and obesity. This year we also had the pleasure of having prominent visiting scientists share their work and an intern learn about our many research projects.

The small staff of Therapeutic Silicone Technologies, Inc.—OFAS's applied research division—is working hard for FDA approval of our medical device to ensure that liquid injectable silicone becomes available to prevent foot amputations caused by diabetic foot ulcers.

We are excited to announce that we are creating new ways to translate our valuable scientific knowledge into lay format in order for the public to appreciate practical advances in aging research. For example, our September 2009 *VitaLongevity* newsletter communicated the potential use of a low methionine diet in extending lifespan. We will continue this effort in our 2010 publications and on our website.

On behalf of the dedicated staff at OFAS, we thank you for your continued support and faith in our mission. We wish you the best in 2010 and look forward to connecting with you through our communications.

Norman Orentreich, MD, FACP
Founder and Co-Director

David S. Orentreich, MD
Co-Director

2009 Research Projects

Clearance of Excess Methionine

Although methionine restriction (MR) is easy to implement in a controlled laboratory environment, this diet presents challenges in everyday life because it requires more planning, preparation, and/or more mindful choices to avoid proteins, especially animal proteins. For nearly three years, we have been developing an easier, 'people friendly' approach for clearing excess dietary methionine using our classic MR model, the Fischer 344 rat. Using a naturally occurring substance that can be taken as an oral supplement or in food, we have observed reductions in body weight and beneficial changes in serum and endocrine chemistries comparable to that of classic MR. In the upcoming year, OFAS hopes to augment its database on this new intervention by continuing to monitor serum and tissue biomarkers, disease incidence, and longevity in these animals.

MR & the Mammalian Target of the Rapamycin Pathway

The mechanism by which MR decreases fat accumulation and extends lifespan in rodents is unknown. Possibly, MR controls processes inside the cells that are involved not only in the utilization of nutrients such as glucose and fat but also in lifespan. One intracellular process extensively reported to control cell metabolism and lifespan involves a protein named the mammalian target of rapamycin (mTOR). Activation of mTOR controls cell growth and division, cell survival, and protein synthesis. mTOR also controls cellular responses to insulin and growth factors and has been implicated in human diseases, especially in various forms of cancer. We are currently examining whether changes in mTOR activity are responsible for the observed MR effects in rats.

Cysteine Supplementation of MR Diet

A Norwegian study using human subjects revealed a direct correlation between total plasma cysteine levels and adiposity. Because cysteine is a metabolite derived from methionine, we conducted collaborative work with Dr. Helga Refsum at Oxford University to examine whether there was a correlation between total serum cysteine and body composition in MR rats. MR reduced total cysteine levels in rats, correlating with reduced adiposity in these animals. Furthermore, the addition of cysteine to the MR diet reversed MR's effects on fat mass, providing strong evidence that adiposity is controlled by blood levels of cysteine.

MR & Breast Cancer

In addition to extending lifespan and reducing age-related pathologies in rodents, MR also causes changes in circulating hormones and growth factors implicated in the promotion of breast tumors. We are currently conducting studies to evaluate the potential use of MR in breast cancer prevention using a rat model that develops breast tumors similar to those observed in humans. This research will provide data that could support or refute the beneficial use of MR diets in breast cancer prevention as well as provide insight about potential anti-breast cancer molecular targets.

MR in Mice: Metabolic Effects, Gene Expression Analysis, & Longevity

MR and calorie restriction (CR) have each been shown to enhance longevity in male Fischer 344 rats. Animals maintained on these dietary interventions have lower body weight, less fat accretion, improved insulin sensitivity, and reduced incidence of age-related disease. For several years, we have been evaluating MR in a hybrid mouse strain (B6C3F1) shown to respond to the life-extending benefits of CR. Survival is significantly increased in females exposed to MR beginning at 12 weeks of age. The results from the male cohort are pending; however, the trend is toward increased longevity in the MR group. Studies addressing MR's metabolic effects are in progress and future plans include evaluating the molecular effects of early, sub-chronic, and long-term MR. Interestingly, compared to rats, this strain of mouse requires a more stringent level of MR to increase lifespan. While this finding agrees with results obtained from other groups using different mouse strains, our studies differ in that the level of methionine is further reduced without adverse effects on animal health.

Transformation of White Adipose Tissue to Brown Adipose Tissue by MR

One observed effect of MR in rats is decreased fat mass. Reduced fat mass in MR rats was proposed to be a response to increased energy utilization specifically in fat depots, and possibly through the increase of mitochondrial content and activity. Cellular and molecular analysis confirmed this hypothesis and also showed that the increased energy expenditure was not restricted to fat tissue but was also observed in other tissues. The ability of MR to increase mitochondrial activity might be important for humans since a feature of obese humans is decreased mitochondrial function in muscle and fat tissues.

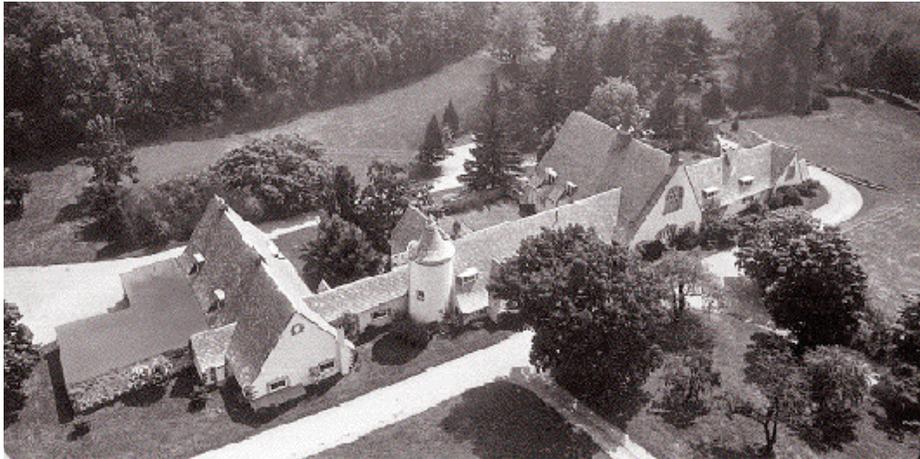
Thyroid Hormone, MR, & Adipogenesis

Increased mitochondrial function and energy utilization could be induced by hormones such as thyroid hormone or adiponectin (secreted by adipose tissue), both of which are increased in rats by MR. MR's role in increased metabolism of thyroid hormone was examined using hypothyroidic F344 rats. It was found that thyroid hormone did not play a significant role in increasing mitochondrial activity in these rats.

2009 Research Projects

Non-alcoholic fatty liver disease (NAFLD) occurs commonly in obese and diabetic populations. An estimated 40 million Americans have some form of NAFLD, and several studies have shown that more than half of obese adults are afflicted. NAFLD often presents as simple fatty liver (hepatic steatosis) and can progress to a more severe condition, non-alcoholic steatohepatitis (NASH). Approximately 15-30% of patients with NASH develop cirrhosis. In addition to insulin resistance and hyperglycemia, genetically obese mice develop hepatic steatosis spontaneously. Our initial observations indicate that MR prevents the development of hepatic steatosis in these animals. We will conduct further studies to validate these initial observations and to explore the biochemical and molecular mechanisms that might explain the hepatoprotective effects of MR in this model.

MR Protects Obese Mice from Hepatic Steatosis



The OFAS Biomedical Research Station in Cold Spring-on-Hudson

2009 Serum Treasury Research

ST Denotes studies using samples from the OFAS-KP Serum Treasury.

ST Half of sudden cardiac mortality occurs in patients with no previous diagnosis of cardiovascular disease. A simple blood test that accurately measures inflammatory protein markers in serum has been developed. It uses a multiplexed ELISA technique (on a Luminex platform) to measure 45 biomarkers from 150 μ l of previously frozen serum. Subjects in the study were identified as participants in the Kaiser Permanente Multiphasic Health Checkup. Serum from healthy subjects was obtained and frozen between 1985 and 1991. This study demonstrated the feasibility and utility of a multiplexed proteomic based algorithm for the prediction of AMI. *Collaborators: Vangelis Hytopoulos, Bruce Phelps, Ted McCluskey, WuXiong Li, Jing Huang (Aviir); Carlos Iribarren, Charles Quesenberry, Jeanne Darbinian (Kaiser Permanente Division of Research); Norman Orentreich, Joseph H. Vogelman (OFAS)*

Multiplexed Proteomic Biomarker Measurement is Prognostic for Acute Myocardial Infarction (AMI)

ST This study examines the association of organochlorides, aflatoxin and hepatitis C with the subsequent development of liver cancer. It is currently in the pilot phase measuring aflatoxin in stored serum from the Serum Treasury. *Collaborators: Stephen K. Van Den Eeden (Kaiser Permanente Division of Research); Christopher Wild (International Agency for Research on Cancer); Joseph H. Vogelman (OFAS)*

Liver Cancer & the Prevalence of Organochlorides, Aflatoxin, & Hepatitis C

ST This study examines the ability of predicting future breast cancer likelihood from the measurement of serum proteins. The method development phase has now been completed. *Collaborators: Yansen Xiao, Edward Nieves, Yee Kai Yeung, Peicheng Du, Mindy S. Ginsberg, Ruth H. Angeletti, Richard Stanley, Thomas E. Rohan (Albert Einstein College of Medicine); Laurel Habel (Kaiser Permanente Division of Research); Joseph Vogelman, Norman Orentreich (OFAS)*

Serologic Biomarker Discovery for Human Breast Cancer Using Proteomics Approach: Method Development

ST This nested case-control study of non-Hodgkin's Lymphoma will be carried out within a cohort of the Northern California Region Kaiser Permanente Medical Care Program. It will include the analysis of a variety of organochlorine levels, EBV seropositivity, and cytokine and immunological biomarkers. This project is composed of two studies taking place in parallel, with samples being analyzed from both studies at three separate analytical laboratories. *Collaborators: Centers for Disease Control and Prevention; National Institutes of Health; Virolab, Inc.; Kaiser Permanente Division of Research; OFAS*

NIH Nested Case-Control Study of Non-Hodgkin's Lymphoma

Accepted for Publication

Sulfur amino acids in methionine-restricted rats: hyperhomocysteinemia.
Elshorbagy AK, Valdivia-Garcia M, Refsum H, Smith AD, Mattocks DAL, Perrone CE.
Journal of Nutrition

Methionine restriction effects on mitochondrial biogenesis and aerobic capacity in white adipose tissue, liver, and skeletal muscle of F344 rats.
Perrone CE, Mattocks DAL, Jarvis-Morar M, Plummer JD, Orentreich N.
Metabolism: Clinical and Experimental

ST Association of pre-diagnostic serum vitamin D levels with the development of basal cell carcinoma.

Asgari MM, Tang J, Warton EM, Chren M-M, Quesenberry CP, Bikle D, Horst RL, Orentreich N, Vogelmann JH, Friedman GD.
Journal of Investigational Dermatology

In Progress

Methionine restriction delays aging-related urogenital diseases in male Fischer 344 rats.
Komninou D, Malloy VL, Krajcik RA, Rivenson A, Orentreich N, Richie JP.

In collaboration with Pennington Biomedical Research Center

Dietary methionine restriction affects metabolic flexibility and uncoupled respiration in both fed and fasted states.

Hasek BE, Stewart, LK, Henagan TM, Boudreau A, Lenard NR, Black C, Shin J, Huypens P, Malloy V, Krajcik RA, Orentreich N, Gettys TW.

Role of the β 3-adrenergic receptor in the hyperphagic and hypermetabolic responses to dietary methionine restriction.

Plaisance E, Henagan TM, Echlin H, Boudreau A, Lenard NR, Hasek BE, Stewart LK, Orentreich N, Gettys TW.

Dietary methionine restriction alters substrate utilization in metabolic syndrome through a mechanism independent of improvements in insulin sensitivity.

Greenway F, Plaisance E, Boudreau A, Liu L, Johnson W, Rood J, Perrone CE, Orentreich N, Cefalu W, Gettys TW.

In collaboration with Kaiser Permanente Division of Research

ST Multiplexed proteomic biomarker measurement is prognostic for acute myocardial infarction.

Iribarren C, Quesenberry C, Hytopoulos V, Phelps B, McCluskey T, Li W-X, Huang J, Darbinian J, Orentreich N, Vogelmann JH.

Poster Presentations

Serum sulfur amino acids in methionine-restricted rats.
Elshorbagy AK, Valdivia-Garcia M, Smith AD, Refsum H, Mattocks DAL, Perrone CE.
7th International Conference on Homocysteine Metabolism. Prague, Czech Republic, June 21-25, 2009.

2002-2009 blueberry study report: Data sets for individual participants now exceed 99.99% confidence for memory, decision speed, and math skill changes.
Doiron DL, Burns DJ, Raphaelson HA, Hull K, Joseph JA, Kokesh AC, Kristal BS, Pruchnicki A, Sachs BS, Schnoll R, Vogelmann JH, Wetherell A, Martin RJ.
38th Annual Meeting of the American Aging Association. Scottsdale, AZ, USA. May 29-June 1, 2009.

Blood glucose negatively correlates with measures of biological age and outcomes of acute neurologic injury in nondiabetics.
Martin RJ, Vogelmann JH, Kristal BS.
38th Annual Meeting of the American Aging Association. Scottsdale, AZ, USA. May 29-June 1, 2009.

Predictive power of spinal cord injury and other clinical data sets may be increased several times by inclusion of prescription and comorbidity data.
Martin RJ, Ratan RR, Vogelmann JH.
2009 Congress on Spinal Cord Medicine and Rehabilitation, Dallas, TX, September 23-26, 2009.

Methionine Restriction—Aging & Disease Seminar

In September, John P. Richie, Jr, PhD, gave a lively two-hour seminar at OFAS dense with methionine restriction (MR) research history and exciting future opportunities. Robust discussion followed.

Dr. Richie, Professor of Public Health Sciences & Pharmacology at Penn State University College of Medicine (Hershey, PA), directs a research program on aging and cancer to elucidate critical factors regulating cancer risk during aging and to develop strategies to prevent cancer and aging-related diseases and disorders. Mechanisms under investigation include pathways involved in the generation of and protection against oxidative stress. Results point to the essential role of glutathione, the major antioxidant in cells and tissues, against aging-related impairments that can lead to cancer development.

Collaborating with OFAS, Dr. Richie and his colleagues have helped to identify dietary MR as an important new means of inhibiting the formation of chemically-induced tumors in the colon. Following up on original findings by Orentreich, *et al.*, that MR could greatly extend longevity in the rat¹, Richie's team found that MR also led to reduced oxidative stress and enhancement of the glutathione system^{2,3}. They also found that MR could eliminate the occurrence of chronic nephropathy and inhibit testicular cancer, two common diseases in older rats.

Altogether, these results indicate that MR is a powerful tool for delaying the aging process. Indeed, leading gerontologists are taking advantage of this novel nutritional approach, evidenced by the upsurge in research publications on the topic. In continuing collaboration with OFAS, the Richie lab is now engaged in studies aimed at identifying specific mechanisms by which MR inhibits oxidative stresses involved in disease development. Further studies are underway to determine the feasibility and efficacy of MR in humans.

References:

1. Orentreich N, Matias JR, DeFelice A, Zimmerman JA. Low methionine ingestion by rats extends life span. *J Nutr* 1993;123:269-74.
2. Richie JP, Jr., Leutzinger Y, Parthasarathy S, Malloy V, Orentreich N, Zimmerman JA. Methionine restriction increases blood glutathione and longevity in F344 rats. *FASEB J* 1994;8:1302-7.
3. Richie JP, Jr., Komninou D, Leutzinger Y, et al. Tissue glutathione and cysteine levels in methionine-restricted rats. *Nutrition* 2004;20:800-5.



Summer Intern's Report

For many years, OFAS has invited undergraduate students to participate in a summer research program. Past interns now have careers in biomedical research or medicine. This year, we were pleased to have Ms. Megan Zerba join the Biology Laboratory staff. Megan is a Biology major at Juniata College. Her valuable assistance allowed us to make significant progress in both the excess methionine clearance and obese mouse hepatic steatosis studies. It was a pleasure to work with Megan. We all agree that she has a bright future ahead of her in biomedical research. Here is part of her report.

"My mentor, Virginia Malloy, allowed me to be so much more involved than I could have hoped for at any other internship. I was exposed to real situations where organization and complete understanding of what you are doing are essential. [I learned] that research is not only about what results you do obtain but also about what results you don't and why. This internship fortified my decision to pursue a biomedical research career thanks to the depth to which I was allowed to participate, the support of everyone I met, and, most of all, the feeling of satisfaction it gave me to know that I can do something to improve the world by making the time we have in it much more enjoyable."—Megan Zerba, Class of 2011, Juniata College (Huntingdon, PA)



Virus Infection—Breast Cancer Seminar



Photo courtesy of Dr. Holland

This March James F. Holland, MD, Distinguished Professor of Neoplastic Diseases at Mount Sinai School of Medicine (NY, NY) gave a vibrant seminar on the topic that most engages him and his research collaborators: the association of a virus with the development of breast cancer. The virus-breast cancer link dates to the 1930s when a virus was found in mouse mammary tumors (MMTV)—a virus that could produce mammary tumors when inserted into healthy mice. Research over the decades has produced solid evidence of a virus-breast cancer link in humans, with Dr. Holland and colleagues publishing in 2007 on the 85-95% similarity between the mouse (MMTV) and human (HMTV) viruses.

He says “We hypothesize that HMTV is a major cause of human breast cancer. Because in time this will cause a seismic shift in concept, we must prove it unambiguously. We have already fulfilled many of the criteria of proof: the virus is present in the neoplasm and not in unaffected tissues; the incidence of the cancer roughly correlates with the frequency of infection; and we have proven the human virus to be infectious. We must now prove that the infection occurs before cancer develops and that infection produces neoplastic behavior in the infected cells.”

To this end, Dr. Holland and colleagues are now analyzing stored sera from the Nurses’ Health Study at Harvard to look for antibodies to HMTV in sera before study participants developed (or controls who did not develop) breast cancer. Because the incubation period is unknown, the Serum Treasury can be enlisted in this important research to provide samples of prediagnostic sera predating those available through the Nurses’ Health Study.

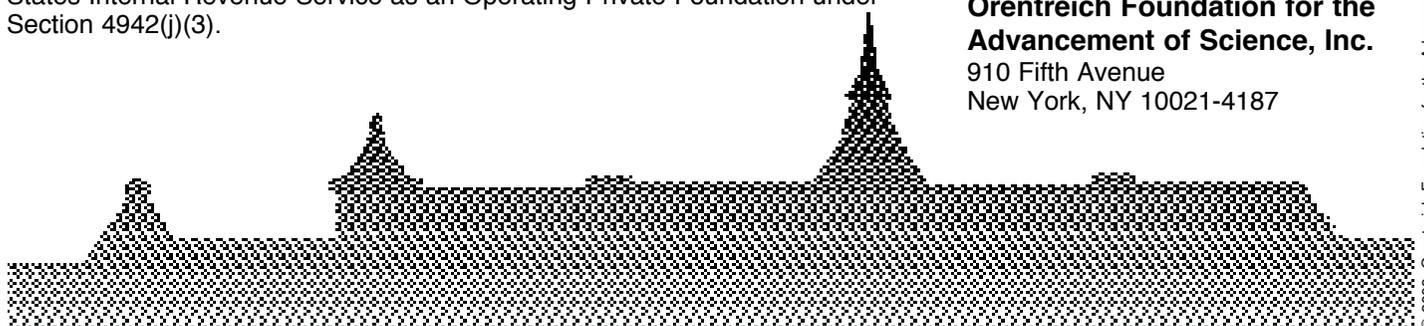
Utilizing the Serum Treasury, OFAS has a significant publication history relating previous viral or bacterial infection to subsequent disease, and the topic of our June 2006 issue of VitaLongevity was “Infections and ‘Unrelated’ Diseases”. Go to <www.orentreich.org> for more and relevant details.

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, 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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Biomedical Research Station

Cold Spring-on-Hudson, NY