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2012
• Collaborative study with Oxford University and the University of Oslo
• Investigation of dietary influence on fatty liver disease
• Initiative to create energy-efficient laboratories

2013
• Review publication: Metabolic adaptations to methionine restriction that benefit health and lifespan in rodents
• First OFAS Symposium: Methionine Restriction & Lifespan
• Collaborative study with Penn State School of Medicine

2014
• Clinical Trial with Penn State School of Medicine
• Collaborative studies with Cornell University and National Chen Kung University (Taiwan)
• Investigation of nutrition and microbiota
• Study of cardiac response to dietary restriction

2015
• Second Symposium: Diet, Sulfur Amino Acids, and Healthspan
• Collaborative study with Yale University
• Initiation of study of methionine restriction in yeast

2016
• Contributing sponsor: 13th International Symposium on Neurobiology and Neuroendocrinology of Aging
• Healthspan Blog launched
• Collaborative studies with Harvard University, Duke University, and Maine Medical Center Research Institute
• Joint conference with The New York Academy of Sciences: Aging and Nutrition: Novel Approaches and Techniques
2016 marks the 55th anniversary of OFAS and its ongoing contributions to biomedical research. OFAS has come long way since its founding in 1961. The last five years have been exciting and impactful as we continue to expand not only our knowledgeable staff and their research but also the means by which we communicate our discoveries. With the addition of new senior scientists and research associates, we have been able to broaden our research on cancer and methionine restriction (MR). We will soon complete our clinical trial examining the metabolic effects of MR in humans, begun in 2014 in conjunction with Penn State School of Medicine. In partnership with The New York Academy of Sciences and The Sackler Institute of Nutrition Science, we co-sponsored a symposium on “Aging and Nutrition: Novel Approaches and Techniques.” Next year we will also host our third biennial OFAS Symposium, bringing together leading researchers in the field of healthy aging to share current investigations and plan future explorations. Finally, 2016 saw the launch of the Healthspan blog and our presence on Facebook and Twitter, all accessible from our website (www.orentreich.org).

The variety of our studies over the past several years is reflected in our peer-reviewed publications on the effects of MR on bone structure, weight loss, obesity and insulin resistance, cell migration, cardiac function, breast cancer prevention, and lipid/glucose metabolism. With every passing year, we broaden our scope and expand the depth of our research in order to pursue our mission of developing interventions that prevent, halt, or reverse disorders that decrease the quality or length of life.

On behalf of the dedicated staff at OFAS, we wish to express our profound gratitude to our generous supporters and wonderful collaborators without whom OFAS’s many milestones over the years would not have been possible. We wish you the best in 2017 and look forward to sharing our newest discoveries with you.

Norman Orentreich, MD, FACP
Founder and Co-Director

David S. Orentreich, MD
Co-Director
Mimicking Methionine Restriction

Methionine restriction (MR) is one of only a few dietary manipulations that have been demonstrated to robustly extend lifespan in mammals. For example, rodents fed a methionine-restricted diet are up to 45% longer-lived than otherwise identical animals fed a normal diet (Orentreich, N et al., 1993; Richie, JP et al., 1994). However, the mechanisms underlying this extension have remained elusive. To address this, Senior Scientist Dr. Jay Johnson used novel cell systems that he had previously developed to model the benefits of MR in the budding yeast S. cerevisiae, as well as in cultured mouse and human cells. He found that manipulations that produce the methionine-restricted state robustly extend the lifespan of yeast and mammalian cells. Specifically, both dietary MR and impairment of the cell’s ability to synthesize methionine (“genetic MR”) significantly extend the lifespan of yeast (Figure 1). Furthermore, genetic MR has been shown to extend the lifespan of mammalian cells (Johnson and Johnson, 2014).

In the last year, Dr. Johnson has identified two genes that underlie the extended lifespan of methionine-restricted cells. They encode key factors involved in autophagy, a process that recycles extraneous or dysfunctional cellular components. Consistent with this finding, Dr. Johnson has also obtained evidence from recent genetic studies that indicates that the primary benefit of MR to yeast lifespan extension is the activation of autophagy. Usefully, he has found that a reduction of cellular methionine levels, as mediated by treatment with the methionine-destroying enzyme L-methionine gamma lyase, produces the methionine-restricted state (P-MR) and extends yeast lifespan (Figure 2). L-methionine gamma lyase administration therefore represents not only a powerful tool for the study of MR, but an intervention that might one day be used to improve human healthspan.

To date, Dr. Johnson has identified a total of six novel interventions that robustly extend cellular lifespan, many of which may ultimately be translatable to humans. In the coming year, his laboratory will continue to identify and characterize interventions that mimic MR, and determine whether they can provide MR-like benefits to mice fed a normal, methionine-containing diet. It is anticipated that these studies will facilitate the eventual development of novel pharmacologic interventions that might be used to improve healthy lifespan in humans.

Figure 1. Genetic MR (A) and dietary MR (B) robustly extend the lifespan of yeast.

Figure 2. L-methionine gamma lyase treatment (P-MR) extends yeast lifespan.
Alterations in Bone Morphology

Associate Science Director Dr. Gene Ables and his team recently found that MR affects the growth rates of both young and aged males and of young females, but not of aged females (Ouattara, A et al., Bone Reports 2016). Previous studies showed that mice on MR had smaller, weaker bones as compared to control-fed (CF) mice of the same age. However, body weight-adjusted parameters indicate that the effect of MR in reducing volumetric bone mineral density, bone mineral content, and microarchitecture measurements is either attenuated or reversed, suggesting that the smaller bones found in methionine-restricted mice are appropriate to their body size. Notably, CF and MR mice had similar intrinsic strength properties as measured by hardness tests (Figure 3). A potential mechanism was identified in a concurrent cell culture study. Mouse preosteoblast cells, precursors to the osteoblast cells that form bone, were cultured in low-sulfur amino acid (SAA) growth media. Under these conditions, gene expression markers for bone formation were downregulated (in low-SAA vs. CF), suggesting delays in collagen formation and the differentiation of preosteoblasts into osteoblasts. Our findings suggest that MR-altered bone morphology could be mediated by these delays in osteoblast differentiation.

Senior Scientist Julie Hens examined the effects of MR on bone morphology in young male mice fed MR and CF diets. MR was found to reduce all aspects of bone structure (density, surface area, etc.), as well as to induce bone marrow fat accretion. Biomechanical testing suggested that bones of mice on MR were extrinsically reduced in strength. Reduced expression of a particular gene (RUNX2) occurred in the marrow of MR mice, the result of elevated levels of several microRNAs (RNA fragments; miRNAs) that interfered with its ability to function. By altering the expression of RUNX2 in bone, these miRNAs could inhibit the number and function of osteoblasts, cells essential to bone formation. Therefore, by increasing miRNAs in bone that can target RUNX2, MR alters bone remodeling.

MR and Breast Cancer

In collaboration with Dr. Raghu Sinha’s lab at Penn State College of Medicine, we studied MR in relation to breast cancer. Dr. Sinha’s lab conducted experiments on the effects of dietary MR on breast cancer tumor progression using a breast cancer xenograft model in which human cells were injected into mice. Mammary tumors of mice on the MR diet were smaller than those in CF mice. Tumors and mammary gland tissue from methionine-restricted mice displayed decreased cell proliferation and increased apoptosis. Decreased cell proliferation corresponded with elevated expression of both human and mouse protein coding gene CDKN1A in mammary gland tissue of MR mice. At OFAS, Dr. Hens’ team performed in vitro experiments, in which upregulation in expression of CDKN1A and CDKN1B genes was observed in both breast cancer cell lines employed (MCF10A and MDA-MB-231) and a decrease in proliferation was seen in MDA-MB-231 breast cancer cells that were grown in MR cysteine-depleted media. Collectively, MR hinders cancer cell progression by increasing cell cycle inhibitors, leading to cell cycle arrest and increased apoptosis. Dietary MR might be of significant interest to breast cancer patients who have decreased levels of genes CDKN1A and CDKN1B.

Figure 3: Hardness of bones in CF and MR mice.
Source: Bone Reports 2016, 5: 33-42.
Aging

Protecting Function of Aging Cells

A characteristic feature of aging is decreased ability to maintain normal cellular functions, which eventually manifests as disease. Optimal maintenance of DNA methylation (the addition of methyl groups to the surface of DNA) is essential for normal cellular functions, but, as organisms age, the number of methyl groups on DNA decreases. This may result in the development of diseases such as cancer, impaired glucose metabolism, and impaired lipid metabolism (Bergman, Y et al., 2013).

The two major dietary sources of methyl groups in animals are methionine and the vitamin choline. Surprisingly, rats and mice fed diets low in methionine (MR) but optimal in choline are less susceptible to aging-associated diseases and outlive mice on regular diets (Ables, GP et al., 2012; Orentreich, N et al., 1993). Therefore, we hypothesized that an MR diet induces changes in DNA methylation, which ultimately contributes to its beneficial effects. Senior Scientist Dr. Sailendra Nichenametla investigated this hypothesis in mice of two different ages (young and adult) by feeding each either a control diet (normal levels of choline and methionine; CF) or an MR diet (normal choline levels, but 80% less methionine).

Findings from this study (Mattocks, DAL et al., 2016) suggest that an MR diet, despite containing fewer methyl groups, prevents loss of DNA methylation in liver. The reason for this paradox appears to be that the MR diet may not be critically low in methyl groups due to the presence of choline. However, the MR diet results in lower levels of S-adenosylhomocysteine (SAH), a biochemical intermediate that inhibits DNA methylation. Previous studies found that SAH levels increase during aging (Stramentinoli, G et al., 1977). Accordingly, we found that adult mice on MR had higher levels of DNA methylation and lower levels of SAH than those on CF (Figure 4A), but MR had no effect on DNA methylation in young mice, likely due to the fact that young animals already have lower levels of SAH compared to adult mice (Figure 4B).

![Figure 4. Effect of MR diet on A) DNA methylation and B) SAH levels. Young (8-week-old) and adult (1-year-old) mice were fed either control diet (CD) or MR diet (MR) for 12 weeks. Error bars are not shown. p-values <0.05 were considered as significantly different.](image)

Senior Research Associate Miri Park preparing samples for analysis.
**Regulation of Adiponectin by MR**

In addition to extending lifespan, MR prevents obesity and improves insulin sensitivity. Previous studies have established that the hormone adiponectin alleviates such detrimental metabolic conditions in several animal models. Rodents on an MR diet show increased levels of plasma adiponectin. Therefore, we hypothesized that the effects of MR are regulated by adiponectin. Dr. Ables is currently leading his team in this investigation using adiponectin-deficient mice fed control or MR diets. Results will be reported in the coming year.

**Obesity & Walkability**

This year, to complement the nutritional aspects of obesity long studied by OFAS, Deputy Director Dr. Bernardita Calinao launched a new project focusing on physical activity as an essential component for the prevention and amelioration of obesity. The project objectives are: 1) to assess sidewalk walkability through microscale research integrated with geographic information systems (GIS) technology and 2) to identify sidewalk improvement opportunities to increase walkability. Walkability is a measure of how friendly an area is to walking, determined by such factors as safety, comfort, beauty, interest, etc. Conducting a microscale assessment of the physical features of the sidewalk, she and her team have investigated a section of the Central Harlem neighborhood of New York City, which has an obesity and overweight rate of 60%. Data generated, assessed, and spatially analyzed will serve as objective indicators of walkability and will help to identify opportunities for place-making, a community-based approach to the planning, design, and management of public spaces in order to create public spaces that promote health, happiness, and well-being. Dr. Calinao was invited to present her work at Walk21, the 17th Annual Conference on Walking and Liveable Communities.

To display the results of this study, a web-based application was developed using ArcGIS Online Software (Figure 5). Color-coded maps reveal whether each street segment is rated as more or less walkable. Open source data, such as the locations of bus stops, restaurants, and trees, are added to the study data to give a fuller picture of the neighborhood.

*Figure 5. Screenshot of the web viewer showing relative walkability of sidewalks in the study area.*
Clinical Nutrition Study in Collaboration with Penn State College of Medicine

For over a century, scientists have attempted to elucidate the mechanisms of the biological aging process and develop practical anti-aging strategies. An important step in this pursuit was our discovery that dietary methionine restriction (MR) was effective at delaying the aging process and enhancing healthspan in laboratory animals. By feeding MR diets to laboratory animals, substantial increases in maximal life-span were achieved. MR was also associated with reductions in aging-related disease processes and disorders. While the mechanisms of MR remain under intense investigation, we have learned that decreased intake of both sulfur amino acids methionine and cysteine is required. In addition, MR effects are associated with dramatic and beneficial changes in the metabolism of fats and carbohydrates and the generation of oxidative stress associated with energy production. It is important to note that the effects of MR are not a response to reduced food consumption; indeed, caloric intake adjusted for body mass is greater in MR rats than in control-fed rats. From what we have learned to date, MR has great potential as a disease preventive/anti-aging intervention for humans.

Thus, an important goal of our research is to translate our findings in laboratory models to determine the potential effectiveness of MR in humans. To this end, we started a controlled feeding study of dietary sulfur amino acid restriction in healthy adults. We hypothesize that the short-term feeding of diets low in the sulfur amino acids methionine and cysteine will result in changes in metabolic endpoints consistent with enhanced longevity, similar to that observed in laboratory animals. These include reductions in the levels of blood lipids, insulin, IGF-1, and leptin; reductions in levels of biomarkers of oxidative stress; and increases in blood adiponectin and fibroblast growth factor 21 levels.

Our current research questions are: (1) what is the most effective level of MR or methionine and cysteine restriction (M/CR) effecting changes in relevant health-related biomarkers; and (2) which biomarkers are affected by M/CR alone and which are responsive to both MR and M/CR. To this end, twenty healthy volunteers have been randomized into either a methionine restricted (MR) group or a methionine and cysteine restricted (M/CR) group (Figure 6). Each group was subjected to three 4-week feeding periods. Blood, urine, and fecal samples were collected at the beginning and end of each period and are being analyzed for relevant biomarkers as described above. Characteristics of study subjects recruited into the study are described in Table 1.

![Figure 6. Study design.](image-url)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Gender (M/F)</th>
<th>Age (yr) mean ± SD (range)</th>
<th>BMI (kg/m²) mean ± SD (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A - MR</td>
<td>10</td>
<td>4/6</td>
<td>38.1 ± 14.9 (24 – 29)</td>
<td>24.8 ± 3.0 (18.8 – 31.5)</td>
</tr>
<tr>
<td>B - MR</td>
<td>10</td>
<td>5/5</td>
<td>37.5 ± 14.7 (25 – 63)</td>
<td>27.5 ± 4.9 (19.1 – 32.8)</td>
</tr>
</tbody>
</table>
To explore the interplay between aging, nutrition, and metabolism, as well as the important role that novel technologies will play in current and future studies, OFAS and The Sackler Institute for Nutrition Science at The New York Academy of Sciences (NYAS) joined together to present a conference on Aging & Nutrition: Novel Approaches & Techniques. A panel of six preeminent researchers in these fields was brought together to discuss the many powerful new techniques being developed that can be used to great effect for studies of healthy aging. The conference was held on December 2 at The New York Academy of Sciences, 7 World Trade Center, New York, NY.

Ellis Rubenstein, NYAS President and CEO, and Dr. Bernardita Calinao, Deputy Director of OFAS, gave the opening remarks. The first session, which focused on new approaches in aging research, was facilitated by Dr. Lenore Launer of the National Institute on Aging, National Institutes of Health. Dr. Jay Johnson of OFAS moderated the second session, which focused on new techniques. OFAS Associate Science Director Dr. Gene Ables and Dr. Gilles Bergeron, Senior Vice President of The Sackler Institute, gave the closing remarks.

Arlan Richardson, PhD, University of Oklahoma Health Sciences Center:
“We have identified a number of genes whose expression is potentially altered by dietary restriction (DR) immediately after the implementation of DR and whose expression remains altered after returning the mice to an ad libitum diet. We identified 5 genes that showed large changes in expression after 1 month of DR and also continued to show these same changes when the mice were fed ad libitum for 2 months.”

Nicholas Stroustrup, PhD, Harvard University:
“How do interventions targeting specific molecular mechanisms produce the beneficial, systemic effects in aging that we observe?
My recent work in *C. elegans* highlights one way in which targeted molecular interventions could produce systemic effects, independent of any single molecular mechanism, depending instead on general principles of complex interdependent networks.

**Matt Kaeberlein, PhD, University of Washington:**

“The goals of the Dog Aging Project are to understand the environmental and genetic determinants of healthy aging in companion dogs and directly intervene in the aging process to improve healthspan in dogs. We believe that results from this project will advance our understanding of the interaction between mTOR signaling and basic aging processes in dogs living in the human environment and could potentially extend the healthspan and lifespan of dogs.”

**Vera Gorbunova, PhD, University of Rochester:**

“Our goal is to identify mechanisms that allow exceptionally long-lived animals to live long and healthy lives and then use these mechanisms to benefit human health. We are undertaking comparative study of aging at the molecular level, exploiting the natural diversity of lifespan across mammalian species to understand the mechanisms of longevity.”

**Vadim Gladyshev, PhD, Brigham and Women’s Hospital, Harvard Medical School:**

“Reduced methionine intake can extend lifespan in rodents by mimicking dietary restriction, but whether this regimen represents a general strategy for regulating aging has been controversial. We found that methionine restriction can extend lifespan of fruit flies and yeast, and this effect is dependent on the status of other amino acids. It is our hope that a better understanding of molecular mechanisms of mammalian lifespan control will lead to a better understanding of human diseases of aging.”

**Jan van Deursen, PhD, Mayo Clinic:**

“Using transgenic mouse models that selectively kill p16ink4a-positive cells, we have investigated the role of senescence in health and lifespan of normal mice, as well as its role in common age-related diseases. The implications of these studies for the design and effectiveness of senotherapies to extend healthy lifespan were discussed.”
OFAS - New York Academy of Sciences Joint Conference

Networking Sessions

Ellis Rubenstein, Lenore Launer, Gene Ables

Reception following the conference

Above: Networking break

Below: Manhattan skyline from NYAS lobby
Staff

Marie Rusin, GIS Specialist
Diana Cooke, Senior Research Associate
Dwight Mattocks, Senior Research Associate
Sailendra Nichenametla, Senior Scientist
Jay Johnson, Senior Scientist, and Jason Plummer, Research Associate


15. Ables GP, Perrone CE, Orentreich D, Orentreich N. Methionine-restricted C57BL/6J mice are resistant to diet-induced obesity and insulin resistance but have low bone density. *PLoS ONE* 2012, 7(12): e51357. doi:10.1371/journal.pone.0051357.


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. In the coming year, we will host the third biennial symposium; this year’s topic will be Healthy Aging.

The keynote address will be given by Dr. George Martin, Professor Emeritus, University of Washington Department of Pathology and Director Emeritus, Alzheimer’s Disease Research Center.
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.

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