First International Mini-Symposium on Methionine Restriction & Lifespan

September 8 – 10, 2013
Tarrytown House Estate & Conference Center
Tarrytown, NY
First International Mini-Symposium on Methionine Restriction & Lifespan

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.
Symposium

Monday, September 9, 2013, 8:30 am – 5:30 pm
Knickerbocker Room, Biddle Mansion

Morning Session
Moderator: Jay A. Zimmerman, PhD

AM 8:30 – 8:40 Welcome – David Orentreich, MD, Co-Director
     8:40 – 8:55 Introduction – Jay Zimmerman, PhD, St. John’s University/OFAS
     8:55 – 9:35 Keynote Address – George Roth, PhD, GeroScience
     9:35 – 10:05 Amany Elshorbagy, PhD, Oxford University
     10:05 – 10:35 Holly Brown-Borg, PhD, University of North Dakota
     10:35 – 10:50 Break
     10:50 – 11:20 Martha Stipanuk, PhD, Cornell University
     11:20 – 11:50 James Mitchell, PhD, Harvard School of Public Health
     11:50 – 12:20 Gene Ables, PhD, OFAS
     12:20 – 1:20 Lunch

PM 1:20 – 1:50 Tsang-hai Huang, PhD, National Cheng Kung University
     1:50 – 2:20 Vadim Gladyshev, PhD, Harvard Medical School
     2:20 – 2:50 John Richie, PhD, Penn State University/OFAS
     2:50 – 3:20 Richard Miller, MD, PhD, University of Michigan
     3:20 – 3:35 Break
     3:35 – 4:05 Rochelle Buffenstein, PhD, University of Texas Health Science Center
     4:05 – 4:35 Blanka Rogina, PhD, University of Connecticut Health Center
     4:35 – 5:05 Chris Church, PhD, Harwell Science & Innovation Campus
     5:05 – 5:30 Review – Joel Brind, PhD, Baruch College/OFAS

*Moderators: AM – Jay A. Zimmerman, PhD;  PM – Arthur J.L. Cooper, PhD
Dr. George S. Roth was formally affiliated with the National Institute on Aging from 1972-2004. After receiving a BS in Biology from Villanova University (1968) and a PhD in Microbiology from Temple University School of Medicine (1971), followed by post-doctoral work with Dr. Richard Adelman at the Fels Research Institute, he progressed from Staff Fellow, to Research Chemist, to Chief of the Molecular Physiology and Genetics Section, to Acting Chief of the Laboratory of Cellular and Molecular Biology. Dr. Roth then served as Senior Guest Scientist at NIA from 2000-2004 and became CEO of GeroScience Inc. He also served as Co-executive Director of the American Aging Association from 2002 to 2003. His research interests continue to be basic mechanisms of aging. He has worked in the area of signal transduction for many years and now focuses on “anti-aging” strategies. The most visible projects in this area have been an examination of the effects of dietary caloric restriction in nonhuman primates and, more recently, the development of caloric restriction mimetics.

Dr. Roth has published nearly 400 papers and a book for lay readers, *The Truth about Aging; Can We Really Live Longer and Healthier?* (Windstorm Creative, 2005). He has served on many editorial boards and has received a number of honors and awards. These include the Sandoz (now Novartis) Prize for Gerontological Research, the Research Award of the American Aging Association, Chair of the Gordon Conference on the Biology of Aging, Chair of the Biological Sciences Section of the Gerontological Society of America, the Merit Award and Equal Employment Opportunity Award of the National Institute on Aging, and the Third Age Award of the International Association of Gerontology. In addition, he has been the Sigma Xi Scholar in Residence at Miami University; an NIH Visiting Professor at Meharry Medical College and the University of Puerto Rico Medical School; the Ben Cohen Memorial Lecturer at the University of Michigan; Keynote Lecturer at the Nagoya International Symposium on Aging and Health and the Israel Endocrine Society; and Alpha Omega Alpha Professor at the University of Puerto Rico. Dr. Roth has mentored 2 PhD students and more than 20 post-doctoral fellows, is frequently interviewed by the media on gerontological research issues, and is listed in *Who's Who in America* and *Who's Who in the World*. 
Sulfur amino acids and body composition
Amany Elshorbagy 1,2, A. David Smith 1, and Helga Refsum 1,3
1Department of Pharmacology, University of Oxford, Oxford, UK
2Department of Physiology, Faculty of Medicine, University of Alexandria, Alexandria, Egypt
3Department of Nutrition, Institute of Basic Medical Sciences, Faculty of Medicine, University of Oslo, Oslo, Norway

Emerging evidence from knockout studies points to involvement of sulfur amino acids in regulation of body composition. Homozygous deletion of cystathionine beta-synthase, which initiates cysteine synthesis from homocysteine, reduces body fat in mice. Mice with a genetic defect in glutathione synthesis have increased energy expenditure and resist obesity. A similar phenotype is observed in rats fed methionine-restricted diets. Common to these models is decreased cysteine synthesis and/or plasma total cysteine (tCys) and profound hepatic suppression of the key lipogenic enzyme stearoyl-coenzyme A desaturase-1 (SCD1).

Supplementation of cysteine, but not taurine, reverses the methionine restriction-induced SCD1 suppression and restores fat gain in rats. In mice, high cystine intake lowers energy expenditure and up-regulates lipogenic and diabetogenic enzymes. In humans, plasma tCys correlates with estimated SCD activity and fat mass. Among upstream compounds, only S-adenosylmethionine shows similarly strong associations with fat mass. In adipocytes, cysteine inhibits lipolysis. Pilot drug studies in mice show that changing cysteine concentrations can favorably influence body composition.

Conclusion
Experimental and epidemiologic data suggest a role for sulfur amino acids in regulation of energy metabolism, in part via an influence on SCD1. Recent reviews of the subject are listed below.

Growth hormone and methionine: Interactions in aging and longevity
H.M. Brown-Borg
School of Medicine & Health Sciences, University of North Dakota

Endocrine hormones impact aging and aging processes in multiple ways. Circulating growth hormone (GH) affects not only somatic growth but also drives aspects of metabolism. We have previously shown that GH modulates methionine metabolism in GH-deficient mice. Restricting methionine (MET) in rodent diets has been shown to lower IGF1 and extend lifespan. Our current studies focus on delineating the relationships between dietary methionine, plasma GH status and factors involved in stress resistance. Our working hypothesis is that GH is involved in the regulation of thiol metabolism that in turn, affects an organisms’ resistance to stressors and ultimately impacts lifespan. Ames dwarf, GH transgenic, and respective wild type mice were subjected to dietary methionine restriction or enrichment. Following eight weeks of MET diets, components of the glutathione and methionine metabolic pathways were examined. Plasma IGF1 levels declined with decreasing dietary MET content. Gene expression of MET conserving and catabolizing enzymes was differentially affected by dietary MET level. Underlying growth hormone status also influenced the metabolic responses to altered dietary methionine. Lifespan studies using Ames dwarf and GH transgenic animals subjected to diets restricted or enriched with methionine are currently underway. At this point, wild type mice on each of the three MET diets show significant increases in median lifespan whereas dwarf mouse median lifespans are unaltered by diet. The results to date suggest that the level of circulating GH interacts with dietary methionine and alters metabolism and lifespan in mice.
Regulation of cysteine dioxygenase in response to sulfur amino acid intake: Is minimizing H₂S production the goal?
Martha H. Stipanuk
Cornell University, Ithaca, NY

The sulfur of sulfur-containing amino acids eventually makes its way to inorganic sulfur or taurine as metabolic end-products that can be excreted in the urine. Cysteine is metabolized by both a direct oxidation pathway, in which the thiol group of cysteine is oxygenated prior to further catabolism to either sulfate or taurine, and by desulfhydration-oxidation pathways, in which the reduced sulfur is released from the carbon chain as H₂S/HS⁻ or sulfane sulfur prior to its further step-wise oxidation to sulfate. Cysteine flux through these pathways is a function primarily of the activity of cysteine dioxygenase (CDO), which initiates flux of cysteine through the direct oxidative pathway, and of cysteine concentration, which is a large determinant of flux through desulfhydration pathways as well as of CDO abundance and activity. CDO is robustly regulated in response to cysteine availability, suggesting that control of cellular cysteine levels is critical. Studies with Cdo1 knockout mice suggest that upregulation of CDO in response to cysteine availability serves to prevent the production of excess levels of H₂S/HS⁻ when sulfur amino acid intake is high. Mice lacking CDO metabolize excess cysteine by desulfhydration pathways, leading to high exposure of tissues to endogenously produced H₂S/HS⁻. These mice exhibit postnatal growth deficits and connective tissue pathologies, but they also exhibit a lean phenotype, being resistant to diet-induced obesity/insulin insensitivity. Future studies will be aimed at defining the beneficial and harmful effects of elevated H₂S/HS⁻ exposure as well as effects of the lack of hypotaurine/taurine.

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

Dietary restriction (DR), defined as reduced food intake without malnutrition, extends longevity when applied for long periods of time in experimental organisms. However, short-term DR lasting only one week can precondition against clinically relevant stressors, such as ischemia reperfusion injury seen as a frequent complication of cardiovascular surgery. Previously, we showed that removal of protein or specific essential amino acids (tryptophan, leucine, or methionine) could precondition against surgical stress in a mouse model of renal ischemia. We also demonstrated a genetic requirement for the amino acid deprivation sensing kinase, GCN2. Here, we found that calorie restriction and essential amino acid restriction can contribute additively to the benefits of DR against surgical stress. An increase in amPK activity and decrease in mTORC1 activity correlated with functional benefits. These findings have translational implications for evidence-based dietary recommendations before elective surgery and other forms of acute stress.
Metabolic effects of dietary methionine restriction in mice
Gene P. Ables
Orentreich Foundation for the Advancement of Science, Inc., Cold Spring, NY

Dietary methionine restriction (MR) in rodents extended lifespan and protected them from developing obesity and diabetes. Since the recruitment of adipose tissue macrophages is implicated in the metabolic syndrome, we asked whether MR reduces its accumulation. To test this hypothesis, lean and diet-induced obese (DIO) mice were fed isocaloric high fat diet (HFD) containing either 0.86% (CF) or 0.12% methionine (MR).

MR mice on HFD had lower body weight despite increased food intake. These mice were more insulin sensitive with reduced hepatic triglyceride accumulation. These mice had higher plasma levels of adiponectin and FGF21, while leptin, SAA, and IGF-1 levels were reduced. The hepatic genes of the MR mice showed the downregulation of Scd1, while Pparg, Atgl, Cd36, Jak2, and Fgf21 were upregulated. The smaller perigonadal adipose tissue (PGAT) in MR mice showed lower gene expressions for Emr1, Cd68, Ccr2, Itgam, and Tnfa and a decrease in F4/80 protein staining.

The DIO-MR mice exhibited weight loss despite increased food intake. These mice became more glucose tolerant and insulin sensitive with lower fasting blood glucose, plasma insulin, leptin, resistin, and PAI-1 and higher adiponectin levels. PGAT, subcutaneous and brown adipose tissue mass were reduced in the DIO-MR mice. PGAT genes showed decreased Ccl2 expression, while Atgl was increased in the DIO-MR mice. Staining for F4/80 in the PGAT showed decreased expression in the DIO-MR mice, which also had smaller adipocyte size.

Overall, our data suggest that MR protects mice from obesity and diabetes with concomitant reduction of adipose tissue macrophage accumulation.
Effects of methionine-restricted diet and endurance exercise training on bones in male growing rats
Tsang-Hai Huang¹, Jack L. Lewis², Hsin-Shih Lin¹,³, Liang-tong Kuo¹, Shih-Wei Mao⁴, Yuh-Shiou Tai⁵, Ming-Shi Chang⁶, Gene P. Ables⁷, Carmen E. Perrone⁷, and Rong-Sen Yang⁸
¹Institute of Physical Education, Health and Leisure Studies, National Cheng Kung University, Tainan, Taiwan ²Department of Orthopaedic Surgery, University of Minnesota, Minneapolis, USA, ³Department of Physical Education, National Taiwan Normal University, Taipei, Taiwan ⁴Department of Mechanical Engineering, R.O.C. Military Academy, Kaohsiung, Taiwan, ⁵Department of Civil Engineering, R.O.C. Military Academy, Kaohsiung, Taiwan, ⁶Department of Biochemistry and Molecular Biology, National Cheng Kung University, Tainan, Taiwan, ⁷Orentreich Foundation for the Advancement of Science, Cold Spring-on-Hudson, New York, USA, ⁸Department of Orthopaedics, National Taiwan University Hospital, Taipei, Taiwan.

Purpose: To investigate the effects of methionine-restricted (MR) diet and endurance exercise (EXE) on growing bone. Methods: Experiment 1: Young male SD rats were assigned to the 0.86%MET, 0.52%MET, and 0.17%MET groups fed with diets containing different levels of methionine (MET) for ten days. Experiment 2: Animals were assigned to six groups fed with similar three MET diets combined with or without EXE for 8 wk. Results: In both experiments, the 0.17%MET fed groups showed significant reduction in body weight, longitudinal growth, and trabecular bone volume. Significant down-regulation of osteocalcin was shown in the 0.17%MET combined EXE group. Serum bone resorption markers were significantly down-regulated due to 0.17%MET feeding and/or EXE in both experiments. Dynamic histology analyses revealed a significant reduction in cortical and/or spongy bone formation rates in 0.17%MET fed animals. And, EXE revealed significant down-regulation in osteoclast density. Both MR diet and EXE caused lower bone mineral content (BMC) measurements, but total BMD of 0.17%MET fed rats was significantly higher in experiment 1 and lower in 0.17%MET plus EXE group of experiment 2. Femora of 0.17% MET fed rats revealed significantly lower whole bone strength, but no difference (experiment 1) and stronger (experiment 2) in tissue-level mechanical properties (e.g. stress, toughness, and elastic modulus). Moreover, EXE provided an enhancement effect in tissue-level properties (e.g. yield stress and toughness) for the 0.86%MET fed animals. Conclusion: MR diets and EXE showed down-regulated effects on bone/energy metabolic indices, bone mass, and/or whole bone strength without further compromising intrinsic bone mechanical properties.
Understanding control of lifespan through genome analyses and methionine status
Vadim N. Gladyshev
Brigham and Women’s Hospital, Harvard Medical School

Understanding the mechanisms that control lifespan is among the most challenging biological problems. Many complex human diseases are associated with aging, which is both the most significant risk factor and the process that drives the development of these diseases. The aging process can be regulated during evolution. For instance, mammals are characterized by >100-fold difference in lifespan, which can both increase and decrease during evolution. We employ this diversity in mammalian lifespan and the associated life-history traits to shed light on mechanisms that regulate species lifespan. For this, we utilize methods of comparative genomics to examine genomes of short- and long-lived species and carry out analysis of lifespan across a panel of mammals. We sequenced the genomes of mammals with most exceptional lifespan, including the naked mole rat and the Brandt’s bat, and identified genes that may contribute to their longevity. These studies point to both lineage-specific and global adaptations involving various pathways. One pathway that emerges as relevant to the control of lifespan is methionine availability. Indeed, 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 could extend lifespan of both fruit flies and yeast, but this effect was dependent on the status of other amino acids. Under certain conditions, methionine restriction mimicked the effect of dietary restriction and was associated with decreased reproduction, whereas under other conditions, it was ineffective, and the regulation of lifespan was uncoupled from reproduction. These studies provide insights into the roles of methionine in aging and suggest a strategy for lifespan extension by methionine restriction. 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.
Previous findings in rodent models that dietary methionine (Met) restriction (MR) increases maximum lifespan and reduces the development of aging-related impairments suggest that MR may have important implications as a preventive or therapeutic strategy in humans. However, to date, there have been few studies aimed at translating these pre-clinical findings to the clinic. To this end, we conducted a short-term controlled cross-over feeding study of MR in healthy adults. This study consisted of 2 isocaloric diet groups (control and 86% MR). Our objectives were to determine the feasibility of feeding an MR diet and to assess the effects of MR on relevant blood biomarkers. The study was conducted with 12 healthy adults and consisted of two 3-week experimental feeding periods (with a 2-week washout). The MR diet was well-tolerated by all subjects with no negative side-effects reported. Decreases in plasma levels of Met (22%) and cysteine (Cys; 15%) were observed in the MR group after 3 weeks. MR significantly decreased plasma total cholesterol (15%), LDL (23%), and uric acid (25%), but had no effects on leptin, adiponectin, IGF-1, or glutathione. Altogether, these findings demonstrate the feasibility of a MR diet in humans and indicate that MR has significant short-term effects on blood lipids similar to those observed in laboratory animal models. In addition, the lack of effects on blood adipokines and glutathione are consistent with more recent laboratory findings that indicate that restrictions in both Met and Cys are required for the full range of beneficial effects on adipokines and longevity.
Drugs, diets, genes that extend mouse lifespan: Any common pathways?
Richard A. Miller
University of Michigan, Detroit, MI

The last 20 years have produced convincing evidence that maximum lifespan in rodents can be increased by at least two diets, mutation of at least half a dozen genes, and at least one drug, rapamycin. Evidence that the longevity effect reflects slower aging, rather than, say, anti-cancer effects on their own, is very strong for caloric restriction and fairly strong for methionine restriction, several of the mutations that block GH and IGF-1 signals, and rapamycin. These new research tools allow initial studies of an interesting question: are there “common pathways” that are altered in parallel in each of these nominally different approaches for slowing the aging process? This presentation will focus on two candidate mechanisms: (a) induction of enzymes that lead to detoxification of xenobiotic and endogenously generated metabolites and (b) activation of ATF4, a protein that senses blocks to translation. The talk will also present data on four newer approaches to lifespan extension in mice: (a) the Crowded Litter (CL) model, based on transient early life nutritional deprivation; (b) acarbose, a drug that slows digestion of starches to sugars and extends lifespan principally in males; (c) nordihydroguaiaretic acid, NDGA, an anti-inflammatory agent that extends male lifespan; and (d) MIF-KO, a mutation that lowers levels of Migration Inhibitory Factor, a pro-inflammatory cytokine with links to insulin secretion and response.
**Stress-enhanced proteostasis in long-lived naked mole-rats**
Rochelle Buffenstein, Kaitlyn N. Lewis, Kelly M. Grimes, and Karl A. Rodriguez
The Sam and Ann Barshop Center for Longevity and Aging Research and Department of Physiology, University of Texas Health Science Center at San Antonio, San Antonio, TX

Improved cellular stress resistance is commonly associated with extended species longevity, although the mechanisms involved remain elusive. It is likely that these include efficient removal of damaged macromolecules. The proteasome is responsible for the eradication of damaged proteins. Declines in proteasome efficiency both with age and in response to stress exposure may play a critical role in age-associated dysfunction of proteostasis. The longest-lived rodents, naked mole-rats, maintain cancer-free good health for 75% of their 32-year lifespan, suggesting that proteostasis is preserved during aging. Mole-rats have higher proteasome activities than do mice. While proteasome activity of mice declines markedly in response to *in vitro* and *in vivo* treatment with oxidative stressors (cadmium and doxorubicin), those of mole-rats increase proteasome activity in response to stress. Mole-rat proteasomes are also resistant to inhibition by proteasome-specific competitive inhibitors (e.g. bortezomib). This is attributed to the presence of a large molecular weight multi-protein protective factor in the cytosol that we have termed “the resistasome”. HSP70 and HSP40 are among constituents of the resistasome; however, ATP hydrolysis is not required for its actions. Co-immunoprecipitation and atomic force microscopy reveal that proteasomes interact with the resistasome. Both mouse and human proteasomes, upon exposure to the mole-rat resistasome, recapitulate the elevated activity and resistance to inhibition observed in mole-rats. Thus, we present evidence for a new transferrable protein assembly that protects mole-rat proteasomes, facilitates their resistance to cytotoxins, and alters their efficiency. This may contribute to the ability of naked mole-rats to successfully defy the aging process.
**INDY mutations maintain fly health and homeostasis**
B. Rogina¹, R.P. Rogers¹, N. Neretti², P.-Y. Wang³, and S.L. Helfand²

¹Genetics and Developmental Biology, University of Connecticut Health Center, Farmington, CT, USA
²Department of Molecular Biology, Cell Biology and Biochemistry, Division of Biology and Medicine, Brown University, Providence, RI, USA
³Neurodegeneration Laboratory, Institute of Neuroscience, National Chengchi University, Taipei City, Taiwan

*Indy* (I’m not dead yet) encodes the fly homologue of a mammalian transporter of di- and tricarboxylate components of the Krebs cycle intermediates. Mutations in the *Indy* gene extend lifespan of the fruit fly. In addition, decreased expression of two of the worm *Indy* homologs extends worm longevity. In flies, INDY is predominantly expressed in places where intermediary metabolism takes place, such as gut, fat body, and oenocytes. Others and our data suggest that *Indy* mutations mimic calorie restriction and extend longevity by related mechanism. This hypothesis is supported by the similarities in physiology of calorie restricted flies with *Indy* mutant flies on high calorie diet, such as lower weight, egg production, levels of triglyceride, decreased starvation resistance, and increased spontaneous physical activity. In addition, *Indy* mutant flies have similar changes in mitochondrial biogenesis as calorie restricted animals. New findings also suggest that *Indy* mutations preserve homeostasis in other tissues that contribute extended health and longevity in *Indy* mutant flies.
The fat mass and obesity associated gene (FTO) regulates body composition and energy homeostasis

Chris D. Church¹, Fiona McMurray¹, Giles S. Yeo², Frances M. Ashcroft³, and Roger D. Cox¹
¹MRC Mammalian Genetics Unit, Harwell Science and Innovation Campus, Oxfordshire, UK,
²University of Cambridge Metabolic Research Laboratories, Institute of Metabolic Science, and National Institute for Health Research Cambridge Biomedical Research Centre, Addenbrooke’s Hospital, Cambridge, UK,
³Department of Anatomy Physiology and Genetics, University of Oxford, UK

Genome wide association studies have identified single nucleotide polymorphisms (SNPs) within the human fat mass and obesity associated gene (FTO) that display strong association with obesity and type 2 diabetes. Individuals homozygous for the at-risk allele weigh, on average, ~3 kg more than individuals with the low-risk allele and display increased food intake, including a preference for fat and protein.

Bioinformatic and in vitro analyses show that FTO codes for a AlkB-like, Fe(II)- and 2-oxoglutarate–dependent nucleic acid demethylase that catalyzes the demethylation of 3-methylthymine, N6-methyladenosine, and 3-methyluracil in single-stranded DNA and RNA. Rodent studies have demonstrated a role for FTO in energy homeostasis and body composition. Mice over-expressing Fto are obese, hyperphagic, and exhibit normal energy expenditure. Conversely, the constitutive germline loss of Fto results in high perinatal lethality and a reduction in body length, fat mass, and lean mass. More recently, we have shown that inactivation of Fto in adult mice leads to reduced body weight, primarily due to a loss of lean mass, and lower RER. Furthermore, selective inactivation of Fto in the hypothalamus, a key site for the nutritional regulation of Fto expression, results in reduced food intake and body weight gain. Additionally, in vitro experiments have identified that Fto expression is dynamically regulated by essential amino acid availability, including glutamine, cysteine, and methionine.

The altered substrate utilization and sensing of dietary macronutrients highlight a critical role for FTO in regulating peripheral metabolism and provide mechanistic insights into how FTO contributes to obesity.