Inflammation in aging and age-related
disease, San Antonio Nathan Shock
Aging Center 2011 Conference on Aging,
Mayan Ranch, Texas Hill Country,
Bandera, TX, USA
Inflammation in Aging and Age-related Disease

The San Antonio Nathan Shock Center Conferences have attracted international speakers and participants since 1995. This annual conference, held in Bandera, Texas, USA, addresses a different topic in the biology of aging each year. The venue's intimate setting, relatively remote location, and common areas are ideal for a small conference (80–100 participants), where copious informal intellectual interchange supplements that of the formal sessions. The 2011 meeting, part of an annual series sponsored by the University of Texas Health Science Center San Antonio, TX, USA, and the Nathan Shock Center of Excellence in the Biology of Aging, addressed the causes of age-associated inflammation and its effect on age-associated diseases.

It is firmly established that aging is associated with chronic low-grade inflammation. Chronic inflammation has been proposed to be a primary cause of aging, to exacerbate/accelerate age-associated diseases such as Alzheimer's disease, to alter normal immune function and thereby increase susceptibility to infection and cancer, and to be a viable pharmacological target for delayed disease progression and enhanced longevity. Age-associated inflammation is the result of a wide range of factors that include deregulation of senescent cells, environmental exposures, and age-associated illnesses. Thus, age-associated inflammation can be considered as both cause and effect during aging at the cellular and organismal level and is inseparable from almost all aspects of biological aging.

The purpose of the 2011 conference was to provide a forum for the presentation and dissemination of recent findings pertaining to age-associated inflammation. To this end, speakers were recruited who are working on exciting new studies that help to clarify the molecular mechanisms underlying age-associated inflammation, and its causal relationship to diabetes, muscle wasting, neurodegenerative diseases, cancer, and infection and immunity, as well as a target for prophylactic intervention. Importantly, in vivo animal models of inflammation during aging were emphasized. Thus, an exciting conference was organized relevant to the understanding of the basis of inflammation during aging, its contribution toward development of age-related diseases, and how anti-inflammatory regimens might confer protection. Abstracts from posters presented at the meeting are presented in this special abstract issue to provide an overview of the breadth and depth of the program. A follow-up issue will publish papers submitted by invited speakers and other participants.

Yoji Ikeno
Carlos Orihuela
Holly Van Remmen
Conference organizers

Abstracts

Inflammation in Aging and Age-related Disease

The San Antonio Nathan Shock Center Conferences have attracted international speakers and participants since 1995. This annual conference, held in Bandera, Texas, USA, addresses a different topic in the biology of aging each year. The venue's intimate setting, relatively remote location, and common areas are ideal for a small conference (80–100 participants), where copious informal intellectual interchange supplements that of the formal sessions. The 2011 meeting, part of an annual series sponsored by the University of Texas Health Science Center San Antonio, TX, USA, and the Nathan Shock Center of Excellence in the Biology of Aging, addressed the causes of age-associated inflammation and its effect on age-associated diseases.

It is firmly established that aging is associated with chronic low-grade inflammation. Chronic inflammation has been proposed to be a primary cause of aging, to exacerbate/accelerate age-associated diseases such as Alzheimer's disease, to alter normal immune function and thereby increase susceptibility to infection and cancer, and to be a viable pharmacological target for delayed disease progression and enhanced longevity. Age-associated inflammation is the result of a wide range of factors that include deregulation of senescent cells, environmental exposures, and age-associated illnesses. Thus, age-associated inflammation can be considered as both cause and effect during aging at the cellular and organismal level and is inseparable from almost all aspects of biological aging.

The purpose of the 2011 conference was to provide a forum for the presentation and dissemination of recent findings pertaining to age-associated inflammation. To this end, speakers were recruited who are working on exciting new studies that help to clarify the molecular mechanisms underlying age-associated inflammation, and its causal relationship to diabetes, muscle wasting, neurodegenerative diseases, cancer, and infection and immunity, as well as a target for prophylactic intervention. Importantly, in vivo animal models of inflammation during aging were emphasized. Thus, an exciting conference was organized relevant to the understanding of the basis of inflammation during aging, its contribution toward development of age-related diseases, and how anti-inflammatory regimens might confer protection. Abstracts from posters presented at the meeting are presented in this special abstract issue to provide an overview of the breadth and depth of the program. A follow-up issue will publish papers submitted by invited speakers and other participants.

Yoji Ikeno
Carlos Orihuela
Holly Van Remmen
Conference organizers
Role of endotoxin in insulin resistance in human muscle

Hanyu Liang¹, Raweewan Lertwattanarak¹, Eugenio Cersosimo¹, Ralph A. DeFronzo², Puntip Tantiwong¹ and Nicolas Musi¹

¹Department of Medicine-Diabetes Division, University of Texas Health Science Center at San Antonio, San Antonio TX, USA; ²The Geriatric Research Education and Clinical Center, South Texas Veterans Health Care System, San Antonio, TX, USA

Emerging evidence has implicated the gastrointestinal flora in the pathogenesis of insulin resistance by causing chronic, low-grade endotoxemia. The goals of this study were to determine: (1) whether obese non-diabetic and obese diabetic subjects have abnormal endotoxin [lipopolysaccharide (LPS)] concentrations in plasma; (2) whether LPS promotes an inflammatory response and induces insulin resistance in human muscle, employing a primary muscle cell culture system. We measured plasma LPS concentrations and insulin-stimulated glucose metabolism (M) with the euglycemic insulin clamp (160 mU/m².min) in 13 lean [BMI = 25 ± 1 kg/m², age = 39 ± 2 years, fasting plasma glucose (FPG) = 92 ± 3 mg/dl, M = 10.4 ± 0.7 mg/kg.min], 9 obese non-diabetic (BMI = 32 ± 1, age = 48 ± 3, FPG = 92 ± 2, M = 8.5 ± 0.7), and 11 obese diabetic (BMI = 34 ± 1, age = 48 ± 3, FPG = 151 ± 13, M = 4.2 ± 0.5) subjects. Obese non-diabetic and diabetic subjects had elevated LPS concentrations in plasma when compared with lean healthy controls. There was an inverse correlation between LPS concentrations and insulin sensitivity (M value). Incubation of human myotubes derived from lean non-diabetic subjects with LPS caused an activation of NFκB signaling in a time-dependent manner, as evidenced by a significant increase in IL-6 and MCP1 gene expressions and phosphorylations of p38 MAPK and Jun N-terminal kinase (JNK). We, then, determined the metabolic consequence of the inflammatory response initiated by LPS in the human myotubes. We found that LPS induced a significant reduction of Akt, AS160, and GSK3 phosphorylations that were correlated with a diminished insulin-stimulated glucose uptake. Interestingly, knocking down toll-like receptor (TLR4), a receptor for LPS, reduced the LPS-induced increases in p38 and JNK phosphorylation as well as IL-6 and MCP1 mRNA expression.

Summary: (1) Obese non-diabetic and obese diabetic subjects have increased concentrations of endotoxin in plasma, and this could play a role in the pathogenesis of insulin resistance; (2) LPS directly causes an inflammatory response that leads to insulin resistance in human muscle; and (3) downregulation of TLR4 blunts the LPS-induced inflammatory response in human muscle, suggesting that pharmacologic blockade of TLR4 could be a useful strategy for the treatment of insulin resistance.

Email: LIANGH0@uthscsa.edu
The expression of MsrA can determine mouse healthspan

Adam Salmon, JennaLynn Styskal, Daniel Pulliam, Yuhong Liu, Holly Van Remmen and Arlan Richardson

Barshop Institute for Longevity and Aging Studies, San Antonio, TX, USA

Inflammation and oxidative stress/damage exist in a cellular yin and yang; the pro-inflammatory state promotes oxidative stress, whereas oxidative damage can induce inflammation at the same time. The interaction of these interconnected, complementary processes may promote many aspects of aging and age-related diseases. Protein oxidation may play a central role in this process as oxidized proteins are potent inducers of inflammation. Oxidation of proteins can also reduce their function, promote their aggregation, and lead to cellular senescence or apoptosis. One way cells reduce these effects is by repairing oxidized proteins with enzymes such as methionine sulfoxide reductases (Msr), which catalyze the reduction of oxidized methionine residues. MsrA is the primary Msr isoform in mammals and is ubiquitously expressed with cellular content of MsrA localized, approximately 25/75 to the mitochondria/cytosol. In this study, we tested the effects of modulation of MsrA on aging processes using transgenic mice lacking MsrA (MsrA<sup>−/−</sup>), mice overexpressing MsrA in the mitochondria (TgMito_MsrA), and mice overexpressing MsrA in the cytosol (TgCyto_MsrA). Using several different markers, we have determined that modulation of MsrA can have profound effects on physiological processes thought to represent healthspan. For example, modulation of MsrA can significantly alter cellular and whole animal resistance to oxidative stress and can affect the mitochondrial generation of oxidative stress. Our data also suggest that modulation of MsrA affects muscle physiology, as we have found significant differences among transgenic mice in their ability to perform forced treadmill running. Perhaps most directly related to inflammatory processes, transgenic MsrA mice also show significantly different physiological responses to high fat diet-induced obesity. However, genetic modulation of MsrA does not seem to affect the maximum lifespan of mice. These data, then, support an important role for MsrA in regulating mammalian healthspan and the promotion of healthy aging.

Email: salmona@uthsa.edu
Long-lived SURF1−/− mice with mitochondrial complex IV dysfunction exhibit increased insulin sensitivity

Deepa Sathyaseelan, Daniel Pulliam, Yun Shi, Adam Salmon, Lauren Sloane, Nicolas Musi and Holly Van Remmen

Barshop Institute for Longevity and Aging Studies, San Antonio, TX, USA

Preservation of mitochondrial function and reduced generation of reactive oxygen species are correlated with increased lifespan and healthspan. Recent studies in invertebrates and rodents have challenged this paradigm by demonstrating that several mitochondrial electron chain alterations are associated with increased longevity. For example, mice lacking Surfeit locus protein 1 (SURF1), a 30 kDa inner mitochondrial membrane protein essential for the assembly of electron transport chain complex IV (Cytochrome c oxidase, COX), have a 50%–80% decrease in COX activity compared to control littermates, yet increased longevity. Maintenance of mitochondrial function is essential for insulin sensitivity, which is one important correlate of longevity. In this study, we asked whether SURF1 null mice have alterations in insulin sensitivity that might contribute to the increased longevity in these mice. Compared to wild-type littermates, SURF1 null mice displayed reduced body weight that could be attributed to decreased fat mass. This reduction in adipose mass in SURF1 null mice is not due to impairments in lipid storage, rather due to increased lipolysis and fatty acid oxidation as indicated by increased phosphorylation levels of hormone sensitive lipase and acetyl CoA carboxylase. PGC1α and its downstream targets NRF1 and Tfam were elevated in the adipose tissue of SURF1 null mice, indicative of increased mitochondrial biogenesis. Increases in fatty acid oxidation and mitochondrial biogenesis are important correlates of insulin sensitivity. Consistent with this notion, a significant improvement in insulin sensitivity was observed in SURF1 null mice; however, their glucose clearance capacity remains unaffected. A strong upregulation of mitochondrial proteases Lon, ClpP, and mitochondrial Hsp60 (components of the mitochondrial unfolded protein response) was observed in the adipose tissue of SURF1 null mice suggesting the involvement of additional components in insulin sensitivity or longevity. Together, these data demonstrate a novel crosstalk between reduced complex IV activity and mitochondrial biogenesis that causes increased fatty acid oxidation and insulin sensitivity and suggest increased insulin sensitivity as a potential mechanism for increased longevity in these mice.

Acknowledgement

This work was supported by the Ellison Medical Foundation.

Email: sathyaseelan@uthscsa.edu
Protein oxidation may be an important regulator in the development of insulin resistance

Jennalynn Styskal¹,², Adam Salmon¹,², Nicholas Musi² and Arlan Richardson¹,²

¹Barshop Institute for Longevity and Aging Studies, San Antonio, TX, USA; ²University of Health Science Center, San Antonio TX, USA

The accumulation of oxidative damage is a proposed mechanism regulating the aging process and the development of disease. Proteins are sensitive to such oxidative stress that can cause them to accumulate, altering conformational structure, and thus the function, of cellular proteins. Methionine sulfoxide reductase A (MsrA) plays an important role in the antioxidant defense but is unique in that it repairs protein oxidative damage. MsrA reduces methionine sulfoxide residues to non-oxidized methionine, thus participating in the antioxidant defense system of cells specifically by protecting proteins from oxidative stress. We have found that mice that lack MsrA (MsrA⁻/⁻) and mice that over express MsrA (MsrA¹🇬) are phenotypically similar to wild-type (WT) mice under normal conditions, but that MsrA levels can regulate susceptibility to oxidative stress. Because these mice are grossly normal, this suggests that excess methionine oxidation may not occur under these physiological conditions. In vivo, increasing adiposity has been associated with increases in oxidative stress, altered redox signaling, and increased oxidative damage to cellular macromolecules in several disease models, including obesity-induced metabolic diseases. When placed on a high fat (HF) diet, MsrA⁻/⁻ mice become more insulin resistant than WT mice, whereas MsrA¹grese mice are protected from development of insulin resistance. The increase in insulin resistance in MsrA⁻/⁻ mice fed on HF diets correlated with reduced insulin-stimulated signaling in the insulin signaling pathway. We found that HF-fed MsrA⁻/⁻ mice had reduced phosphorylation of both insulin receptor and Akt with administration of insulin under HF fed conditions. Also, increased insulin sensitivity seen in the HF-fed MsrA¹grese mice correlated with an increase in insulin-stimulated signaling in the insulin signaling pathway. These results suggest that oxidative damage, specifically to proteins, may play an important role in obesity-induced insulin resistance.

To address how protein oxidation may cause insulin resistance, we have utilized ex vivo studies to test the effect of MsrA on oxidative stress-induced insulin resistance. By utilizing these models, this study will test the hypothesis that MsrA can regulate the development of insulin resistance by repairing oxidative damage in proteins involved in the insulin signaling pathway in vitro. Insulin resistance can be induced ex vivo by various forms of oxidative stress (H₂O₂ and palmitate). In this study, skeletal muscle isolated from MsrA⁻/⁻, MsrA¹grese, and WT mice was tested for resistance to oxidative stress under these conditions. Insulin-signaling protein phosphorylation correlates with in vivo signaling observations, determined by western blot after insulin stimulation. Our hypothesis is that the level of protein oxidation can be correlated with the degree of insulin resistance in this system. Because oxidation of proteins can lead to a decline in their function, future studies will focus on both function of the insulin-signaling proteins isolated from these models as well as oxidation status of these proteins.

Email: styskal@uthscsa.edu
TAK-242, a small-molecule inhibitor of toll-like receptor-4 signaling, protects against lipopolysaccharide- and lipid-induced inflammation in muscle cells

Sophie Hussey¹,² and Nicolas Musi¹,²

¹Department of Medicine-Diabetes Division, University of Texas Health Science Center at San Antonio, San Antonio TX, USA; ²The Geriatric Research Education and Clinical Center, South Texas Veterans Health Care System, San Antonio, TX, USA

Recent evidence suggests that the lipopolysaccharide (LPS) receptor, toll-like receptor-4 (TLR4), and signaling pathways downstream of TLR4 play a role in skeletal muscle insulin resistance. Much data implicate saturated free fatty acids (FFA), whose circulating levels are often elevated in individuals with insulin resistance, as ligands for TLR4. Our lab recently demonstrated elevated concentrations of LPS in plasma of patients with type 2 diabetes (T2D), providing another mechanism for elevated TLR4-mediated inflammation in these individuals. TAK-242, a small-molecule antisepsis agent, binds selectively to TLR4 and inhibits its downstream signaling events. The purpose of the present study was to investigate the effect of TAK-242 on LPS and FFA-induced inflammatory pathways in skeletal muscle. L6 myotubes were preincubated with/without TAK-242 (1μM) for 1 h, prior to stimulation with 100 ng/ml of LPS for 1 h, or 400 μM stearic acid for 1 and 6 h. LPS caused an inflammatory response, as evidenced by increased phosphorylation of Jun N-terminal kinase (JNK) (2.1-fold), p38 MAPK (5.4-fold), IκBα (3.9-fold) and p65 NFkB (8.4-fold), and reduced IκBα protein levels (5.3-fold). TAK-242 completely inhibited the phosphorylation of these proteins in response to LPS. Stearic acid treatment for 1 h increased phosphorylation of JNK (1.5-fold), IκBα (74%), and p65 NFkB (94%), whereas TAK-242 partially reduced the increase in the phosphorylation of the inflammatory proteins by 22, 62, and 84%, respectively. Stearic acid treatment for 6 h increased phosphorylation of JNK (3.6-fold), p38 (6.6 fold), and p65 NFkB (1.2-fold), and TAK-242 reduced the stearic acid-mediated increases in JNK (by 38%) and p38 (by 51%), although p65 NFkB phosphorylation was not affected.

Summary: (1) TAK-242 reduces LPS- and lipid-induced inflammation in muscle cells; (2) LPS induces an inflammatory response primarily by activating TLR4; and (3) saturated FFA also works through TLR4 to induce an inflammatory response, although other mechanisms likely are involved in this process.

Conclusion: TAK-242 may represent a novel therapeutic approach to reduce inflammation and improve insulin action in conditions associated with insulin resistance such as obesity, aging, and T2D.

Email: Husseys@uthscsa.edu
A novel conserved mechanism of extended longevity in a mouse model of mitochondrial dysfunction

Daniel Pulliam, Deepa Sathyaseelan, Yuhong Liu and Holly Van Remmen

Barshop Institute for Longevity and Aging Studies, San Antonio, TX, USA

Dysfunctional mitochondria can lead to disruptions in ATP production and increase the reactive oxygen species related to aging. Previously, it was thought that dysfunctional mitochondria would lead to a decrease in lifespan; however, mutations or knockdowns resulting in a decrease in the activity of the mitochondrial electron transport chain have been shown to increase longevity in Caenorhabditis elegans, drosophila, and recently mice. However, in C. elegans, disruption of the mitochondrial unfolded protein response (mtUPR) reverses this lifespan extension. The mtUPR is a retrograde signaling response resulting in an increase in mitochondrial specific antioxidants, chaperones, and proteases aimed at refolding misfolded proteins and degrading damaged proteins.

Here, we explore a novel conserved mechanism of longevity in the mitochondrial complex IV-deficient Surf1 knockout mouse. Knockout of Surf1, which codes for a complex IV assembly factor, results in a 50%-80% decline in complex IV activity and a 20% increase in median lifespan. This dysfunction results in tissue-specific differences in superoxide and hydrogen peroxide production, membrane potential, and ATP production. Interestingly, these fibroblasts are more resistant to the superoxide generator paraquat. Here, we show that these mice have an increase in mitochondrial specific chaperones (HSP60) and proteases (Lon and CLPP) that have been linked to mtUPR providing a possible mechanism for the increased resistance to cellular stresses. Taken together, the Surf1 knockout mouse could have enhanced longevity due to a novel mechanism conserved from invertebrates to mammals.

Acknowledgement
This project was supported by a grant from the Ellison Medical Foundation.

Email: PulliamD@uthscsa.edu
Despite sarcopenia, aging does not affect myofiber regenerative capacity in mice

Heather M. Hancock¹, Matthew McHale¹, Laurel Porter¹,², Zaheer Sarwar², Linda M. McManus¹ and Paula K. Shireman¹,²

¹Departments of Surgery and Pathology, The University of Texas Health Science Center at San Antonio, San Antonio, TX, USA; ²South Texas Veterans Health Care System, San Antonio, TX, USA

Introduction: Muscle maintenance is a lifelong process in which myofibers are removed and replaced, a response that is increased after muscle injury. Sarcopenia or the loss of muscle mass occurs with aging. One theory suggests that muscle regenerative capacity declines with age to the point that muscle regeneration cannot keep pace with injury that occurs through daily living activities. Although multiple studies have quantitated sarcopenia in animal models of aging, few reports document recovery from injury in aged animals. We hypothesized that aged mice would exhibit impaired muscle regeneration defined as smaller fiber size and increased adipocyte accumulation as compared to young mice. Thus, the present study examined myofiber size and adipocyte accumulation within regenerated muscle in a murine model of aging.

Methods: Three age groups of wild-type mice (C57Bl/6J) were studied: young (4–6 months old), middle (12–14 months), and old (25+ months) and included males and females (n = 5–13/group). Mice were obtained at 8 weeks of age from Jackson Laboratories. Cardiotoxin (CTX) was injected into the right hind limb muscles to cause muscle injury. After 14 days, the hind limb anterior muscle compartment was harvested, formalin fixed, paraffin embedded, sectioned, and stained with hematoxylin and eosin. Untreated animals (baseline) served as a control. Light microscopic histomorphometry was used to quantitate tibialis anterior myofiber size (cross-sectional area, μm²) and intramuscular adipocyte content (percentage of regenerated muscle area).

Results: At baseline, female mice had smaller myofiber size at all ages as compared to male mice of the corresponding age (see Table 1). Although young and middle-aged mice had similar baseline myofiber size, myofibers were considerably smaller in old animals of both sexes. After injury, regenerated myofiber size was proportionally comparable to that of baseline myofibers in male mice at all ages. Note that at 14 days post-injury, myofiber size did not return to baseline size. Similar patterns of myofiber regeneration were observed in female mice following CTX-induced injury.

Regardless of age, no intramuscular adipocytes were present at baseline in either male or female mice. Following injury, young, middle, and old male mice had comparable increases in adipocyte accumulation within the areas of regenerated muscle (0.99 ± 0.24%, 1.1 ± 0.34%, and 0.95 ± 0.38%, respectively). Females shared a similar pattern of fat accumulation with increased fat as compared to corresponding aged males, i.e. 2.9 ± 0.65%, 4.4 ± 0.49%, and 2.1 ± 0.54%, respectively.

Conclusion: In an age-independent manner, female mice accumulated more adipose tissue within the area of muscle regeneration as compared to males. Nevertheless and despite sarcopenia, aging did not have a significant impact on myofiber regenerative capacity in either male or female mice.
Table 1. Effect of aging on myofiber size before and after injury.

|            | Male       | Female     |
|------------|------------|------------|
|            | Baseline   | 14D post-CTX | Baseline   | 14D post-CTX |
| Young      | 2,628 ± 52.1 | 1,632 ± 101.6 | 2,115 ± 68.4 | 1,624 ± 81.5 |
| Middle     | 2,797 ± 62.7 | 1,523 ± 43.6 | 2,077 ± 60.7 | 1,423 ± 36.9 |
| Old        | 2,090 ± 124.9 | 1,402 ± 75.1 | 1,499 ± 206.5 | 1,326 ± 68.4 |

*aValues expressed as mean ± SE (n = 5 – 13/group).

Acknowledgements
This research was supported in part by the Veterans Administration (Merit Review Grant), the National Institutes of Health (HL074236), and the United States Air Force.

Email: hancockh@uthscsa.edu
Elucidating the chondroprotective function of cartilage ECM protein matrilin-3

Chathuraka T. Jayasuriya¹, Mary B. Goldring², Richard Terek¹ and Qian Chen¹

¹Department of Orthopedics, Warren Alpert Medical School of Brown University, Providence, RI, USA; ²The Hospital for Special Surgery, Weill Cornell Medical College, New York, NY, USA

Osteoarthritis (OA) is a leading cause of disability in the United States, and it afflicts more than 30 million Americans who are past middle age. Currently, there are no FDA approved drugs specific for the treatment of this debilitating age-associated degenerative joint disease. Although not a classical inflammatory arthropathy, OA is frequently associated with signs and symptoms of inflammation, including joint pain, swelling/stiffness that may lead to functional impairment. Furthermore, increased levels of inflammatory cytokines such as IL-1β, either locally in the cartilage or in the synovial fluid during OA pathogenesis, can repress the synthesis of type II collagen (COL2A1) and aggrecan (ACAN) and upregulate proteinases such as matrix metalloproteinases and the disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) family of aggrecanases, thereby ultimately favoring cartilage degradation. We have previously demonstrated that mice lacking the matrilin-3 gene develop OA very early in life. Elucidating the biological mechanism by which MATN3 can delay OA is a critical first step in understanding its potential as a novel OA therapy. In our efforts to do so, we discovered that recombinant human matrilin-3 (rhMATN3) protein can inhibit several catabolic effects of IL-1β (including its downregulation of COL2A1 and ACAN and its stimulation of the major catabolic proteinase, ADAMTS-5). Furthermore, we have also recently obtained strong evidence suggesting that matrilin-3’s regulation of these targets is dependent on its ability to stimulate interleukin-1 receptor antagonist (IL-1Ra); a potent IL-1β pathway inhibitor. Future studies will focus on characterizing the signal transduction pathway involved in matrilin-3 induced stimulation of IL-1Ra.

Acknowledgement
This study is supported by NIH AG 017021, AG 014399, and RR024484.

Email: chathuraka_jayasuriya@brown.edu
ACL transection decreases the residence time of high-molecular weight hyaluronan in synovial fluid

William J. McCarty, Ju C. Cheng, Benjamen C. Hansen, Gary T. Yamaguchi, Koichi Masuda and Robert L. Sah
University of California-San Diego, La Jolla, CA, USA

Introduction: Synovial fluid (SF) contains hyaluronan (HA), an important lubricant for joint articulation at high-molecular weight (Mw) and concentration. After injury and in osteoarthritis, SF HA concentration and Mw decrease, although the biophysical mechanisms for this are unclear.

Hypothesis: HA efflux from SF is higher after injury in a Mw-dependent manner.

Methods: The residence time of HA for various Mw was determined in n = 12 rabbit knees at days 7 (n = 4), 14 (n = 4), and 28 (n = 4) after unilateral ACLT, with IACUC approval, by collecting SF, lavaging with saline, injecting bilaterally with saline or HA (n = 1 or 3 at each time point), and sampling at 1, 3, and 8 hr. Joint fluids were analyzed for HA concentration and Mw, and time constants (τ) were calculated. Data are expressed as mean ± SEM.

Results: After saline injection, high Mw HA accumulated over time, whereas after HA injection, [HA] was lower (1) with decreasing Mw, (2) for ACLT compared to Non-Op knees, and (3) at day 7 compared to 28 (Figs 1, 2). HA residence time in the joint was lower in ACLT than Non-Op control joints at day 7, but not at day 28 (Fig. 3).

Discussion: The decreased HA residence time in the joint at early times after ACLT may be due to the inflammatory response in synovium following surgery and ACLT injury. This study identifies increased efflux of high-Mw HA as a biophysical mechanism of diminished HA concentration after injury and during joint inflammation, as often occurs in osteoarthritis.

Funding
NIH-NIA 1F31AG039939, NIH-NIAM

Fig. 1. fluids: (A) normal rabbit SF, (B-G) saline injection, (H) injected HA, HA injection at days (I-N) 7 and (O-T) 28 after surgery.
Fig. 2. Total HA by M₁ (A) saline injection, (B) injected HA, and after HA injection at days (C) 7 and (D) 28. Stacked bars: (top) endogenous HA and (bottom) injected. Dashed line: normal rabbit SF.

Fig. 3. Calculated time constants by M₁ bin for Non-Op (−) and ACLT (+) data at days 7 and 28.* P < 0.05; ** P < 0.01 for Non-Op versus ACLT.

Email: wmccarty@ucsd.edu, rsah@ucsd.edu
Differential microRNA expression patterns in CCR\textsuperscript{2\textminus/\textminus} versus wild type mice following skeletal muscle injury

David W. Melton, Jonathan A.L. Gelfond, Yongxin Chen, Linda M. McManus and Paula K. Shireman

The University of Texas Health Science Center at San Antonio, San Antonio, TX, USA

Dynamic events in injured skeletal muscle lead to a robust regenerative response that is critically dependent on macrophages; however, the regulatory role of these inflammatory cells remains to be established. Given the important role of microRNA (miRNA) in macrophage biology, it seems likely that these small, non-coding RNAs are involved in macrophage-specific biological events. The present study was designed to explore this possibility by exploiting the known defect in macrophage recruitment and activation in CCR\textsuperscript{2\textminus/\textminus} mice. Thus, the temporal expression of macrophage-related miRNA in muscle regenerative events in vivo was examined in cardiotoxin-injured muscle derived from wild type (WT, C57Bl/6J) or CCR\textsuperscript{2\textminus/\textminus} mice. Total RNA was extracted from muscle and miRNA expression determined by qRT-PCR in TaqMan assays (Applied Biosystems). As compared to baseline (uninjured) muscle, log fold change in miRNA expression at 1, 3, or 7 days following injury (n=4 mice/group/timepoint) was determined. In a separate group of animals studied at similar timepoints, monocyte/macrophage numbers in injured muscle were determined by flow cytometry and confirmed a significant reduction in macrophage infiltration in CCR\textsuperscript{2\textminus/\textminus} animals. In both mouse strains, considerable changes in diverse miRNAs were observed following injury. But, most importantly, in comparisons of the temporal expression patterns of miRNA from WT and CCR\textsuperscript{2\textminus/\textminus} mice, cluster analysis revealed 60 miRNAs with different patterns of expression, including miRNAs that have been reported to be highly expressed in macrophages. Interestingly, 15 miRNAs with no known relationship to macrophages were matched with the temporal expression patterns of macrophage-related miRNAs. Thus, it is conceivable that this latter group of miRNAs may be unique, macrophage-related miRNA that regulate the muscle regenerative response. Given the parallel development of sarcopenia and altered macrophage biology during aging, the miRNAs identified in the present study may be useful in the elucidation of new targets for therapeutic intervention of sarcopenia.

Acknowledgement

Research supported in part by the Veterans Administration (Merit Review Grant) and the National Institutes of Health (F30 HL110743 and R01 HL074236).

Email: meltond@uthscsa.edu

Pathobiology of Aging & Age-related Diseases 2011. © 2011 D. W. Melton et al. This is an Open Access article distributed under the terms of the Creative Commons Attribution-Noncommercial 3.0 Unported License [http://creativecommons.org/licenses/by-nc/3.0/], permitting all non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Citation: Pathobiology of Aging & Age-related Diseases 2011, 1:14729 - DOI: 10.3402/pba.v10.14729
Lovaza® prevents aging-associated bone loss in C57BL/6 mice by inhibiting inflammation and bone resorption

Md M. Rahman, Ganesh Halade, Jyothi M. Veigas, Kazi Nishu, Paul J. Williams and Gabriel Fernandes

University of Texas Health Science Center at San Antonio, San Antonio, TX, USA

Aging-associated bone mineral density (BMD) loss is becoming a significant, worldwide social and financial health care problem. In this study, we demonstrated the beneficial role of FDA-approved prescription omega-3 fatty acids, Lovaza® against BMD loss during aging using 12-month-old C57BL/6 mice. Mice were fed with 1 and 4% of Lovaza® and placebo diets including a group with 4% regular fish oil (18/12 (EPA/DHA)) diet for 12 months. DXA scan was performed once before starting the diet at 12 month of age and again after 12 months of feeding. After analyzing the BMD of different bone regions, we found that 4% Lovaza® prevents age-associated BMD loss, while 1% Lovaza® offers mild protection. However, 1% Lovaza® showed better protection than that of 4% regular fish oil. We also measured the effect of LPS-stimulated cytokines production in bone marrow (BM) cells collected from mice fed with different experimental diets. Interestingly, 4% Lovaza® showed decreased production of pro-inflammatory cytokines, TNF-α, and IL-6 and increased production of anti-inflammatory cytokines IL-10 and IFN-γ as compared to that of other groups. We also analyzed RANKL-stimulated activation of inflammatory and pro-osteoclastogenic signaling molecules p38 MAPK and JNK in BM cells using Fast Activated Cell-based ELISA (FACE) assay system. Interestingly, both 1 and 4% Lovaza® showed reduced activation of both p38 MAPK and JNK. Furthermore, we analyzed the components of bone remodeling in serum samples for TRAP5b, PINP, RANKL, and OPG. Bone resorption marker, TRAP5b level was dramatically reduced in 4% Lovaza® treated mice, whereas bone formation marker, PINP level was unaffected. Moreover, the osteoclast stimulating factor, RANKL was significantly reduced in 4% Lovaza® treated mice, whereas the decoy receptor for RANKL, OPG level was unaffected. These data indicate that Lovaza® protects age-associated bone loss in C57BL/6 mice by modulating inflammatory signaling molecules and related bone resorption.

Email: rahmanm@uthscsa.edu
The role of resveratrol and sirtuin1 in sarco-osteopenia during aging

Kim Seldeen, Martin Pang and Bruce R. Troen

Division of Gerontology and Geriatric Medicine, University of Miami Miller School of Medicine, Miami VA Geriatric Research Education and Clinical Center, Miami, FL, USA

Age-related sarco-osteopenia involves declines in mass, quality, and strength of both muscle and bone. Because the protein deacetylase Sirtuin1 (Sirt1) has been associated with improvements in the healthspan in mice involving both muscle and bone, Sirt1 may be an excellent target for study for its potential in preventing and/or ameliorating sarco-osteopenia. We are currently exploring the effects of resveratrol (RSV), a putative activator of Sirt1, on bone. Our preliminary data demonstrate that in vivo supplementation with RSV dramatically improves bone quality in aged mice, stimulates osteoblastogenesis, and inhibits osteoclastogenesis in young mice, and enhances resistance to oxidative stress in bone marrow cells. Our data suggest a possible mechanism involving downregulation of Rac1 activity and decreased phosphorylation of c-Src, resulting in altered actin polymerization. Given the importance of Rac1-dependent actin reorganization in muscle, we have begun to examine the impacts of RSV and Sirt1 on skeletal muscle mass and performance during aging. To elucidate potential mechanisms of action, we will analyze protein and mRNA transcriptional levels of Sirt1 and proteins involved in inflammatory pathways influencing sarco-osteopenia because Sirt1 appears to play a role in suppressing NF-κB activity. In addition, we will assess the impacts on oxidative stress. Taken together, these studies will shed new light on the interrelatedness of sarco-osteopenia leading to the development of novel strategies for prevention.

Email: seldeen@hotwiremail.net
Determining the functional role of caspase-2 in senile osteoporosis

Danielle A. Victor¹, Ramaswamy Sharma¹, Difernando Vanegas¹, Diane Horn², Kathleen Woodruff², Meenakshi Tiwari¹, Marisa Lopez-Cruzan¹, Sherry L. Werner² and Brian A. Herman¹

¹Department of Cellular and Structural Biology, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA; ²Department of Pathology, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA

Normal bone homeostasis is maintained by balancing osteoclast-mediated bone resorption with osteoblast-induced bone formation. If this delicate balance is chronically tipped toward excess bone resorption, osteoporosis occurs. Age-related osteoporosis in men is of particular importance because mortality following fracture is higher in men than in women. Our lab has previously shown that old male mice deficient in caspase-2 (casp-2), a cysteine protease involved in apoptosis, exhibit severe age-related osteoporosis. Specifically, micro-computed tomography (μCT) and dual energy X-ray absorptiometry analyses of long bones from old Casp-2⁻/⁻ mice demonstrated decreased bone mineral density. Molecular biological analyses revealed that cleaved caspase-2 decreases as a function of age, directly correlating to a decrease in bone density seen in old wild type (WT) mice. In addition to its role in regulating cellular apoptosis, caspase-2 also plays a role in mesenchymal stromal/stem cell (MSC) differentiation to osteoblasts. Indeed, MSCs from Casp-2⁺/⁺ and Casp-2⁻/⁻ mice have decreased mineralization activity compared to WT. In addition, cultured MSC lysates and whole bone lysates from Casp-2⁻/⁻ mice expressed higher levels of CSF-1, a cytokine that enhances osteoclast survival. Together, these data indicate that the function of Casp-2⁻/⁻ osteoblast lineage cells is altered such that bone resorption is favored. Immunohistochemistry of bone sections as well as in vitro culture of osteoclast precursors indicates that caspase-2 is not present in osteoclasts at a basal level but is upregulated in response to mitochondrial oxidative stressors such as rotenone and antimycin A. Therefore, caspase-2 may play a role in osteoclast apoptosis during conditions of mitochondrial oxidative stress, as has been demonstrated in neurons and fibroblasts. Because caspase-2 staining in murine long bone sections and human bone sections is similar, our data are relevant to human studies. Elucidating the function of caspase-2 in bone under normal conditions as well as in age-dependent osteoporosis will lead to novel anti-osteoporosis therapies to aid millions affected by this debilitating disease.

Email: victorD@livemail.uthscsa.edu

Abstracts

Pathobiology of Aging & Age-related Diseases 2011.

#2011 D. A. Victor et al. This is an Open Access article distributed under the terms of the Creative Commons Attribution-Noncommercial 3.0 Unported License (http://creativecommons.org/licenses/by-nc/3.0/), permitting all non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Citation: Pathobiology of Aging & Age-related Diseases 2011, 1: 14729 - DOI: 10.3402/pba.v1i0.14729
Increased bacterial colonization in aged mice is associated with delayed b-defensin induction

Aleah L. Brubaker\textsuperscript{1,3,4,5} and Elizabeth J. Kovacs\textsuperscript{1,2,3,4,5}

\textsuperscript{1}Department of Surgery, Burn & Shock Trauma Institute, Loyola University Medical Center, Maywood, IL, USA; \textsuperscript{2}Department of Surgery, Loyola University Medical Center, Maywood, IL, USA; \textsuperscript{3}Department of Cell Biology, Neurobiology & Anatomy, Loyola University Medical Center, Maywood, IL, USA; \textsuperscript{4}Immunology & Aging Program, Loyola University Medical Center, Maywood, IL, USA; \textsuperscript{5}Stritch School of Medicine, Loyola University Medical Center, Maywood, IL, USA

Advanced age is associated with aberrant inflammation and innate immune cell activation that contribute to refractory responses to trauma, tissue injury, and infection. Despite clinical observations that aging impairs wound healing, few studies have examined how the innate immune response contributes to resolution of cutaneous wound infection. Using an excisional wound injury model, young (3-4 months) and aged (18-20 months), BALB/c mice received $10^2 \times 10^3$ CFU/mL per wound of \textit{Staphylococcus aureus} (\textit{S. aureus}), a common dermatopathogen with a predilection for elderly patients. Over 7 days, aged mice exhibited increased bacterial colonization at early time points with incomplete resolution at day 7 as compared to young mice (two-way ANOVA, $F = 0.1617$; Age, $p = 0.0003$; Time, $p = 0.0376$). To determine the contribution of age-related alterations in the innate immune response to the heightened bacterial colonization seen in aged mice, we examined wound neutrophil and macrophage phagocytosis, as well as antimicrobial peptide expression. Following isolation of wound neutrophils and macrophages, these leukocytes were examined via flow cytometry for phagocytosis using the pHrodo bioparticle system. Although no difference in neutrophil or macrophage phagocytosis was seen between cells from young and aged animals, it was noted that neutrophil expression of FcgRIII (CD16) was decreased following phagocytosis in latter group ($p < 0.001$). As no difference in leukocyte phagocytic potential was observed between young and aged animals, we sought to examine another aspect of the innate immune response, cathelicidins and b-defensins that are induced following tissue injury and infection. Within the b-defensin family, we examined mouse b-defensin 3 (mBD3), mBD14, and cathelicidin-related antimicrobial peptide (CRAMP). In young mice, mBD3 and mBD14 were upregulated at day 1 after injury and infection. However, in aged mice, the upregulation of mBD3 and mBD14 was significantly less than that seen in young mice ($p < 0.05$). No differences were observed in cathelicidin expression between age groups. The lack of mBD3 and mBD14 induction in wounds from aged mice following wound infection may allow time for increased bacterial growth, contributing to heightened susceptibility to bacterial pathogens and delayed resolution of cutaneous wound infection in the setting of advanced age.

Acknowledgements

This work was supported by NIH R21 AI073987 (EJK), R01 AG018859 (EJK), T32 AG031780 (PWL), and Dr. Ralph and Marian C. Falk Medical Research Trust (EJK) and the MD/PhD Program at Loyola University, Stritch School of Medicine.

Email: abrubaker@lumc.edu
Aging-associated H$_2$O$_2$-NOX2 dysregulation in human neutrophils

Amina El Jamali, Anthony J. Valente and Robert A. Clark.

Department of Medicine, University of Texas Health Science Center, and South Texas Veterans Health Care System, Audie L. Murphy Division, San Antonio, TX 78229-3900, USA

Neutrophils comprise the first line of host defense, killing invading microbes by generating reactive oxygen species (ROS) through the activable NADPH oxidase (NOX2) system. This respiratory burst oxidase is genetically absent in patients with chronic granulomatous disease, resulting in recurring severe infections with high morbidity and mortality. The phagocyte oxidase features membrane-bound catalytic components (gp91phox/p22phox) and cytosolic cofactors (p47phox, p67phox, Rac1/2) that undergo stimulus-dependent translocation during assembly of the active NADPH oxidase.

Given the importance of H$_2$O$_2$ as a cellular signaling molecule and our recent finding demonstrating a positive feedback regulation of the NOX5 isoform by H$_2$O$_2$, we explored the possibility that the phagocytic NOX2 system is regulated by H$_2$O$_2$. Our data show that H$_2$O$_2$ induces superoxide production in human blood neutrophils and monocytes, as well as in K562 leukemia cells overexpressing NOX2 system components. We demonstrated that H$_2$O$_2$ stimulates NOX2-mediated superoxide generation in both neutrophils and K562/NOX2 cells via a signaling pathway involving Ca$^{2+}$ influx and c-Abl tyrosine kinase acting upstream of PKCδ. Relevant to the oxidative stress associated with aging, we found recently that this pathway is dysregulated in human neutrophils from healthy older individuals. Among subjects age 20-65, there was a trend for an age-related increase in basal superoxide generation, whereas basal rates in those older than 65 were decreased relative to the younger age groups. In addition, one way ANOVA analysis across all age groups (20-87 years) demonstrated significant age-related declines in the activation levels of NOX2 by H$_2$O$_2$, especially for subjects older than 65.

Thus, dysregulation of phagocyte ROS production over time (i.e. failure to produce appropriate amounts of ROS in the right place at the right time) may contribute to the aging process. Identification of the signaling proteins responsible for abnormal NOX2 regulation by H$_2$O$_2$ will help to explore mechanisms of inflammation-associated aging phenotypes such as increased susceptibility to infection and autoimmunity, delayed phagocyte apoptosis, and chronic inflammation.
Mechanisms contributing to regulatory T Cell homeostasis in aging

Jana Raynor¹, Pulak Tripathi¹, Allyson Sholl¹, David Plas², Claire Chougnet³ and David Hildeman¹

¹Division of Immunobiology, Cincinnati Children’s Hospital Medical Center and Department of Pediatrics at the University of Cincinnati College of Medicine, Cincinnati, Ohio, USA; ²Department of Cancer and Cell Biology, University of Cincinnati, Cincinnati, Ohio, USA; ³Molecular Immunology, Cincinnati Children’s Hospital Medical Center and Department of Pediatrics at the University of Cincinnati College of Medicine, Cincinnati, Ohio, USA

T cell function declines with age and impairs the ability to fight new infections and control persistent infection. We previously showed that regulatory T cells (T⁰reg), an immune suppressive CD4⁺ T cell subset, accumulate dramatically with age in both humans and mice. Furthermore, T⁰reg accrual in aged mice prevented control of persistent infection. Moreover, we found that decreased expression of the pro-apoptotic molecule Bim in aged T⁰reg contributes to enhanced survival and subsequent T⁰reg accrual. Given the role of IL-2 in T⁰reg homeostasis in young animals, we investigated the role of IL-2 on modulating T⁰reg survival and Bim expression in aging. We found that IL-2 is sufficient to promote accumulation of Bimlo T⁰reg in young mice. Also, in aged mice we found that IL-2 neutralization resulted in a partial loss of T⁰reg, but selected for CD25lo T⁰reg. Interestingly, the remaining CD25lo T⁰reg population had increased expression of CD122 (IL-2/IL-15Rβ). Using aged IL-15 deficient mice, we found that IL-15 partially contributed to T⁰reg accrual. Interestingly, we found that the additional loss of Bim (IL-15-/- Bim-/-) rescued the loss of T⁰reg in aged IL-15-deficient mice. Mechanistically, we explored the role of FOXO transcription factors in control of Bim expression in T⁰reg. Notably, we found that deletion of FOXO1/3a/4 in vivo led to significantly decreased Bim expression in T⁰reg. Together, our data suggest that IL-2 and IL-15 contribute to T⁰reg accrual in age and may also contribute to the suppression of Bim expression in aged T⁰reg by signaling through FOXO family members.

Email: raynor@cchmc.org
Overexpression of foxn1 prevents age-associated alterations in thymic epithelial cell architecture and the decline in naïve T cell production

Erin C. Zook, Paulette A. Krishack, Jiwang Zhang, Nancy J. Zeleznik-Le, Anthony B. Firulli, Pamela L. Witte and Phong T. Le

Cell Biology, Neurobiology and Anatomy Graduate Program and Program for Immunology and Aging, Department of Microbiology and Immunology, Stritch School of Medicine, Loyola University Chicago, Maywood, IL, USA

The thymus is the primary organ for thymopoiesis and the production of naïve T cells from early T cell progenitors (ETP). With age the thymus involutes, resulting in a decline in the production of naïve T cells, which contributes to a compromised immune system in the elderly leaving them more susceptible to illnesses and infection. A mechanism for thymic involution and the decline in naïve T cell production with age is not well elucidated. Previously, we showed that a decline in expression of the transcription factor Foxn1 with age correlated with reduced thymopoiesis as determined by the decline in thymocyte number. Using a transgenic mouse model in which Foxn1 is overexpressed under the human keratin 14 promoter (Foxn1Tg), we found that aged Foxn1Tg mice had a higher number of thymocytes compared to Wt. Overexpression of Foxn1 prevented the decline in ETP frequency, and aged Foxn1Tg had a 3-fold higher number of ETP compared to aged Wt. Overexpression of Foxn1 also diminished age-associated changes in thymic epithelial cell (TEC) composition. Foxn1Tg had a higher number of EpCAMpos medullary TEC (mTEC) as well as of the MHCIIhi mTEC; these cells express the highest level of Foxn1 and are identified as transient amplifying cells that are responsible for maintaining the mTEC pool. While the percentage of EpCAMpos MHCIIhi mTEC that is Ki67pos declined with age in Wt, a higher percentage of Ki67pos EpCAMpos MHCIIhi mTEC was found in aged Foxn1Tg. Furthermore, aged Foxn1Tg had more peripheral naïve T cells compared to Wt, and the age-associated expansion of CD4 memory T cells was prevented. Taken together, our data suggest that maintaining high expression of Foxn1 in the thymic environment can prevent age-associated changes in TEC composition leading to improving thymopoiesis and production of naïve T cell with age.

Acknowledgements
This work was supported by NIH R01 AG32809 (PL), R01 AG013874 (PW), T32 AI007508 (EZ), T32 AG031780 (EZ), and an intramural pilot project grant from Loyola University, Stritch School of Medicine (PL).

Email: ezook@lumc.edu
Deficiency of Gpx1 and Aldh1a1 genes is associated with increased lipid peroxidation and motor deficits in young adult mice

Xiang Bai¹,², Margaret Chia-Ying Wey¹,², Anthony Martinez¹,², Phil Bergmen², Vanessa Martinez¹,², Elizabeth Fernandez¹,²,³ and Randy Strong¹,²,³

¹Department of Pharmacology, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA; ²Barshop Institute for Longevity and Aging Studies, University of Texas Health Science Center at San Antonio, TX, USA; ³Geriatric Research, Education and Clinical Center, South Texas Veterans Health Care Network, San Antonio, TX, USA

Parkinson’s disease (PD) is the most prevalent neurodegenerative movement disorder affecting up to 5% of the population aged 65–85 years. PD is characterized by progressive dopaminergic neurodegeneration in the nigrostriatal pathway leading to profound dopamine depletion in the substantia nigra and striatum. Evidence from different labs suggests that oxidative stress plays a role in pathogenesis of PD. Previous studies have shown that expression of ALDH1 and GPX1, which are important for clearance of aldehydes and H₂O₂, respectively, are reduced in the substantia nigra of PD patients. To determine the contribution of deficiency in these two genes to the pathogenesis of PD, our lab has generated mice with simultaneous deletion of Aldh1a1 and Gpx1 genes (Aldh1a1/Gpx1 KO). Young adult Aldh1a1/Gpx1 KO mice showed a significantly increased latency to fall in the automated accelerating rotarod test and increased reversal time in the pole test compared to age-matched wild type control mice. There were no significant effects on levels of dopamine and its metabolites DOPAC and HVA, dopamine transporter or vesicular monoamine transporter 2 protein in midbrain and striatum. However, levels of 4-hydroxynonenal, an end product of lipid peroxidation, were increased in the midbrain and striatum of Aldh1a1/Gpx1 KO. In summary, simultaneous deletion of Gpx1 and Aldh1a1 genes was not associated with altered measures of dopaminergic function but was associated with motor deficits in young adult mice. In addition, deficiency of Gpx1 and Aldh1a1 genes was associated with increased lipid peroxidation. Future experiments will include determining behavioral phenotypes in mice at middle and old ages, evaluating the number of dopaminergic neurons in the substantia nigra. Also, because Gpx1 is predominantly expressed in microglia and neuroinflammation has been suggested to be important for pathogenesis of PD, levels of the microglia marker Iba-1 in substantia nigra and striatum, as well as the number of Iba-1 positive cells in brain, will be determined.

Email: baix@uthscsa.edu
Reduction of mitochondrial H$_2$O$_2$ by Prdx3 overexpression attenuates cognitive impairment and the elevated amyloidogenesis induced by paraquat in an AD mouse model

Liuji Chen$^{1,2}$, Si-Eun Yoo$^{2,3}$, Ren Na$^2$, Yuhong Liu$^{1,2}$ and Qitao Ran$^{1,2,4}$

$^1$Department of Cellular and Structural Biology, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA; $^2$The Barshop Institute for Longevity and Aging Studies, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA; $^3$Department of Physiology, University of Texas Health Science Center at San Antonio, TX, USA; $^4$Geriatrics Research Education and Clinical Center, South Texas Veterans Health Care System, San Antonio, TX, USA

Alzheimer’s disease (AD), one of aging-associated diseases, is the most common dementia affecting millions of people around the world. Epidemiological studies indicate that exposure to environmental toxins, such as pesticides, is a risk factor of AD. However, little is known about how pesticide exposure may promote AD pathogenesis. In this study, we investigated the effects of paraquat exposure on cognition and amyloidogenesis in AD mouse models. Our results indicated that paraquat exposure increased the generation of H$_2$O$_2$ by brain mitochondria, which was directly correlated with increased mitochondrial oxidative damage and reduced mitochondrial function. Moreover, APP transgenic mice exposed to paraquat showed exacerbated cognitive impairment and elevated Aβ levels. Peroxiredoxin 3 (Prdx3) is a key enzyme for mitochondrial H$_2$O$_2$ removal. Our results showed that overexpression of Prdx3 prevented paraquat-induced increase in mitochondrial H$_2$O$_2$ generation and mitochondrial damage. Importantly, both cognitive impairment and elevation of Aβ levels induced by paraquat exposure were significantly attenuated in APP transgenic mice with Prdx3 overexpression. Therefore, our results demonstrate that increased mitochondrial H$_2$O$_2$ generation is a key mechanism by which pesticide exposure exacerbates AD pathogenesis.

Email: chenl2@uthscsa.edu
Naked mole-rat neurodegeneration: is the hippocampus better protected?

Yael H. Edrey¹, Salvatore Oddo² and Rochelle Buffenstein³

¹Department of Cell & Structural Biology, University of Texas Health and Science Center, San Antonio, TX, USA; ²Department of Physiology, University of Texas Health and Science Center, San Antonio, TX, USA; ³Barshop Institute for Longevity and Aging Studies, University of Texas Health and Science Center, San Antonio, TX, USA

Despite more than a century of research, the primary mechanisms involved in the pathogenesis of the most common age-related neurodegenerative disease, Alzheimer's disease (AD), remain unclear. Laboratory mice and rats do not develop this disease, and this is attributed to their short lifespan. We propose an extraordinarily long-lived rodent, the naked mole-rat as a novel, natural model for the study of AD. We assessed common risk factors for AD in this species and surprisingly found that young (2 year) naked mole-rats (NMRs) exhibited many of these risk factors: We found high levels of lipid peroxidation in NMR brains. High oxidative damage is associated with neuronal damage in human AD. We also found that levels of both soluble and insoluble Aβ in the brains of NMRs were comparable or greater than levels found in a genetically manipulated common AD mouse model (3XTg). These findings prompted us to evaluate if specific brain regions commonly associated with AD (cortex, hippocampus) are better protected against these harmful affects than the relatively unaffected cerebellum. Despite their exceptional longevity, young NMRs exhibited high levels of Aβ particularly in the hippocampus and this was coupled with low antioxidant defense and low proteasome activity. These factors might render the NMR hippocampus particularly susceptible to neuronal damage. In addition, we report the unique key properties of NMR Aβ (sequence, aggregation propensity and toxicity) that suggest NMRs may be an important addition to the sporadic AD models studied to date.

Email: edrey@uthscsa.edu
A role for redox-dependent demyelination: implication for inflammation and metalloproteinases

Ryan T. Hamilton, Michael E. Walsh, Yun Shi, Arunabh Bhattacharya and Holly Van Remmen

Barshop Institute for Longevity and Aging Studies and Cellular and Structural Biology, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA

Increasing evidence suggests that oxidative stress plays an important role in neuronal function, and proper neuronal communication is important for muscle physiology. We have previously shown that mice lacking CuZnSOD (Sod1−/−) have an accelerated muscle atrophy phenotype and high levels of oxidative stress and that calorie restriction protects against this loss in both Sod1−/− and wild-type (Wt) mice. We also find a significant decrease in nerve conduction velocity (NCV) in Sod1−/− mice at all ages compared to Wt mice, and calorie restriction (CR) attenuates this loss in the old Wt animals. We hypothesized that oxidative stress plays a role in loss of NCV through demyelination by increases in inflammation and metalloproteinases that converge on myelin function. To test this hypothesis, we used two groups of mice to measure spinal cord and sciatic nerve-dependent biochemical changes: (1) ad libitum-fed C57Bl6 Wt mice at 6, 21, and 29 months of age and calorie restricted (40% restricted) mice at 29 months of age and (2) Wt and Sod1−/− mice at 6 and 21 months of age when significant muscle atrophy is present. We found a significant decrease in sciatic NCV of Sod1−/− mice at all ages, and in 29-month-old Wt mice this reduction was reversed by CR. Myelin compactness and neuronal size are lower in sciatic nerves from mice with increased oxidative stress (Sod1−/− and 29 month Wt) and were reversed by CR at 29 months of age. The observed differences in myelin compactness may support the reduced insulation of neurons and loss of NCV. One potential mediator of this phenotype is inflammation. Sod1−/− mice have elevated MCP1 protein expression as a percentage of the Wt littermate control value ((6 month 187 ± 14.9*) and (20 month 206 ± 13.9*)). This level of MCP-1 expression is equivalent to the level in 29-month-old Wt mice (191 ± 19.9*) and is attenuated in 29 month CR group (130 ± 12.9*). Another marker of inflammation, matrix metalloproteinase-9 (MMP9), expressed as a percentage of the littermate control value, is elevated at all ages in the Sod1−/− mice (6 month (118 ± 5.9*) and 20 month (189 ± 13.9*)) and is dramatically elevated in the 29-month-old AL mice (438 ± 100.9*) and is attenuated by CR (120 ± 8.9*). ERK signaling, a known regulator of MCP-1 and MMP-9, expressed as a percentage of the littermate control value, is equivalent at all ages in the Sod1−/− mice (6 month (118 ± 5.9*) and 20 month (189 ± 13.9*)) and is dramatically elevated in the 29-month-old AL mice (438 ± 100.9*) and is attenuated by CR (120 ± 8.9*). Additionally, these data suggest that a reduction in NCV and myelin may be in part regulated by inflammatory processes related to oxidative stress. Associated with this reduction in NCV and myelin compactness is a concomitant increase in both MCP1 and MMP-9 protein expression in the old wt mice, where oxidative stress is known to peak, and at all ages in the Sod1−/− mice. Further support is a reduction in MCP-1 and MMP-9 with CR and a concomitant attenuation of the age-dependent loss in NCV and myelin compactness. Future studies with MCP1 and MMP-9 knockout mice will be aimed to determine the role of both of these proteins involvement in loss of NCV, myelin compactness, and muscle atrophy with age.

Email: hamiltonrt@uthscsa.edu

Citation: Pathobiology of Aging & Age-related Diseases 2011, 1: 14729 - DOI: 10.3402/pba.v1i0.14729
Regulation of heat shock proteins through mTOR signaling improves cognitive function in mice modeling AD

Anson Pierce²,³#, Natalia Podlutskaya³#, Matthew J. Hart²,⁴, Jonathan J. Halloran¹, Raquel Burbank¹ and Veronica Galvan¹,²*

¹Department of Physiology, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA; ²The Barshop Institute for Longevity and Aging Studies, San Antonio, TX, USA; ³Department of Cellular and Structural Biology, San Antonio, TX, USA; ⁴Department of Molecular Medicine, San Antonio, TX, USA

We recently showed that inhibition of mTOR by systemic, long-term rapamycin treatment blocks AD-like cognitive impairments and Aβ accumulation in mice modeling AD. To determine the mechanisms by which long-term inhibition of mTOR modulates cognitive function, we examined the complete proteome of brains of control- and rapamycin-treated PDAPP mice. Members of the chaperone/heat shock response (HSR) family were overrepresented among the proteins whose abundance was increased by rapamycin treatment in transgenic brains. In agreement with the observed upregulation of heat shock proteins (HSPs), we found that HSF1 was activated, and that eIF4E-biding protein (4E-BP, which participates in shutoff of protein synthesis during heat shock) was activated, setting up conditions for the preferential translation of chaperone mRNAs in rapamycin-treated brains. Overexpression of HSF1 mimicked the effects of rapamycin, resulting in chaperone upregulation and preservation of cognitive function in PDAPP mice. Conversely, inhibition of the chaperone HSP90 strongly increased Aβ levels in transgenic brain slice cultures. Taken together, our results indicate that activation of the chaperone network is sufficient to lower Aβ levels and block cognitive impairments in PDAPP mice and suggest that long-term inhibition of the mTOR pathway may decrease Aβ levels and preserve cognitive function by increasing the levels of chaperones in transgenic mouse brains. Maintenance of proteostasis may, therefore, be sufficient to preserve cognitive function in AD-like neurodegeneration. Our data suggest that rapamycin, already used in clinical settings, may have potential for the treatment of AD.

Email: podlutskaya@uthscsa.edu

²These authors contributed equally to the present work.
Refinement of novel olfactory acuity assay for assessment of health span evaluation

Samantha Rendón¹,², Kathleen E. Fischer¹,³ and Steven N. Austad¹,²

¹Barshop Institute for Longevity and Aging Studies, San Antonio, TX, USA; ²Department of Cellular & Structural Biology, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA; ³Department of Physiology, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA

Decline in sensory acuity is a general hallmark of aging, which in humans decreases quality of life. We report here refinement of a sensory acuity assay in mice. Three features of the assay merit attention. First, as mice are primarily nocturnal in nature, olfaction is an important sensory modality for them. Second, our assay instead of using artificial olfactory cues employs major urinary proteins that are important in both intrasexual and intersexual communication of mice in nature. Third, the assay can be performed in the mouse’s home cage, thus avoiding artifacts from distracting, novel environments. Procedurally, the assay uses serial dilutions of urine and preference for the urinary odor relative to water control to olfactory acuity. Age-related changes in olfactory acuity have not previously been reported in mice. We have refined the assay that compares time spent sniffing a sample relative to time spent at a distilled water control, numerous times, and the most recent refinement appears to be sensitive, repeatable, and encompasses particularly informative urinary dilution ranges. Specifically, previous testing revealed that of any age, mice usually cannot distinguish urine from water at a dilution of 1:10,000 (Rendón, unpublished data). The range of experimental dilutions that have been narrowed down through successive modifications of the assay is between 1:10,000 and 1:5,000. Sampling in this range allows us to pinpoint and compare discriminatory ability and to more accurately assess the influence of factors, such as age, or drugs, on mouse olfactory acuity.

Email: rendons@uthscsa.edu
Effect of the aldehyde trapping agent, hydralazine, in Parkinson’s disease

Margaret Chia-Ying Wey1, Shou-Shu Wang2, Anthony Martinez3, Xiang Bai1, Vanessa Martinez3, Patricia Sullivan4, David S. Goldstein4, Elizabeth Fernandez1,3,2 and Randy Strong1,3,2

1Department of Pharmacology, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA; 2Geriatric Research, Education and Clinical Center, South Texas Veterans Health Care Network, San Antonio, TX, USA; 3Sam and Ann Barshop Institute for Longevity and Aging Studies, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA; 4Clinical Neurocardiology Section, Clinical Neurosciences Program, Division of Intramural Research, National Institute of Neurological Disorders and Stroke, Bethesda, MD, USA

Parkinson’s disease (PD) is an age-associated neurodegenerative disorder affecting 2%-5% of the population 65-85 years of age. PD is characterized by progressive motor dysfunction resulting primarily from death of substantia nigra dopaminergic neurons. Numerous studies indicated that the accumulation of inflammatory and oxidative insults during aging contributes to PD pathogenesis. Biogenic aldehydes can be triggered by inflammation and are particularly toxic due to their reactivity, relatively long half-life, and the fact that they can easily cross cell membranes, acting as second toxic messengers. The accumulation of biogenic aldehydes in brains of deceased PD patients has been extensively reported. Aldehyde dehydrogenases (ALDH) play a major role in detoxification of biogenic aldehydes in the brain. Previously, our lab has shown that mice null for two Aldh isoforms known to be expressed in midbrain dopamine neurons, Aldh1a1 and Aldh2, exhibited age-related deficits in motor performance on the accelerating rotarod and in gait analysis. The deficits were rescued by L-DOPA administration. Consistent with the loss of ALDH, biogenic aldehydes were elevated in the midbrain of the Aldh1a1−/− × Aldh2−/− mice. The lipid peroxidation product, 4HNE, was elevated by 30%-60% in midbrains of Aldh1a1−/− × Aldh2−/− mice. We also found significant (2-6-fold) accumulation of DOPAL, the aldehyde product of dopamine metabolism, in midbrain and striatum of Aldh1a1−/− × Aldh2−/− mice. An increasing number of reports indicate that aldehyde trapping agents may be cytoprotective in conditions of increased ‘aldehyde load.’ Therefore, we hypothesized that aldehyde trapping agents may be beneficial in PD. To test the hypothesis, we first used a PC12 cell culture model and found that Hydralazine prevented 4-HNE-induced cell death. We then delivered Hydralazine (250 mg/L) in drinking water to the Aldh1a1−/− × Aldh2−/− mice. Long-term delivery of Hydralazine (~ 40 mg/kg/day for 9 months) rescued motor performance deficits in Aldh1a1−/− × Aldh2−/− mice. Reduced midbrain 4-HNE level in Aldh1a1−/− × Aldh2−/− mice after long-term Hydralazine treatment supported our hypothesis that aldehyde trapping agent (i.e. Hydralazine) scavenges accumulating aldehydes and may provide a new therapeutic approach to PD. As a currently FDA-approved antihypertensive treatment, Hydralazine may be readily approved as a neuroprotective agent in PD.

Acknowledgements
This work was supported by the VA Office of Research & Development (EF and RS), PHS: AG036613 (RS) and NRSA (NIA) pre-doctoral training grant T32 AG021890-08 (MW).

Email: weym@uthscsa.edu
Gender-specific differences in C57BL/6 mouse sleep fragmentation

Keith Maslin¹,², Keyt Fischer¹, Vanessa Soto¹, Lauren Sloane¹, Stephen Treaster¹,⁴ and Steven Austad¹,³,⁴

¹Barshop Institute for Longevity and Aging Studies, The University of Texas Health Science Center at San Antonio, San Antonio, TX, USA; ²Department of Physiology, The University of Texas Health Science Center at San Antonio, San Antonio, TX, USA; ³Department of Cellular and Structural Biology, The University of Texas Health Science Center at San Antonio, San Antonio, TX, USA; ⁴Department of Molecular Medicine, The University of Texas Health Science Center at San Antonio, San Antonio, TX, USA

Sleep fragmentation is a common health issue in aging populations. Most commonly studied in relation to sleep apnea, it has been demonstrated in humans that sleep fragmentation is associated with disruption of many body functions ranging from glucose metabolism to cognitive function.

In an effort to assess the effects of rapamycin on healthspan in C57BL/6 mice, we used a non-invasive assay to monitor animal activity. The Pack Laboratory developed a high-throughput model for phenotyping sleep in mice, which validates the use of activity/inactivity assessments as a measure of sleep, with sleep defined as any bout of inactivity of ≥40 seconds. Based on their results, we measured activity across a 24-h light/dark cycle to assess sleep fragmentation.

We report here that C57BL/6 mice that were started on rapamycin at 19 months of age show significant sex differences in sleep fragmentation unrelated to aging. Sex differences in sleep fragmentation are also found in aging humans; the mechanism underlying this sex difference warrants further investigation.

Email: Maslin@uthscsa.edu
Healthspan effects of rapamycin on aging C57BL/6 mice, a preliminary assessment

Kathleen Fischer¹,², Vanessa Y. Soto¹, Lauren B. Sloane¹, Samantha Réndon¹,³, Rocío G. Reyes¹, Keith Maslin¹,⁴, Stephen Treaster¹,⁴ and Steven Austad¹,³,⁴

¹Barshop Institute for Longevity and Aging Studies, The University of Texas Health Science Center at San Antonio, San Antonio, TX, USA; ²Department of Physiology, The University of Texas Health Science Center at San Antonio, San Antonio, TX, USA; ³Department of Cellular and Structural Biology, The University of Texas Health Science Center at San Antonio, San Antonio, TX, USA; ⁴Department of Molecular Medicine, The University of Texas Health Science Center at San Antonio, San Antonio, TX, USA

Inhibition of the TOR signaling pathway by rapamycin has been shown to extend measures of lifespan in genetically heterogeneous mice and C57BL/6 mice. To determine whether rapamycin treatment delays age-associated declines in healthspan, we performed a single-blinded study using C57BL/6 mice fed with encapsulated rapamycin or control diet starting at 19 months of age. We tested these mice with a series of putative healthspan assays chosen for their similarity to measures of aging and frailty in humans. Several of the assays showed significant differences between rapamycin-treated animals and controls. In some cases, rapamycin-treated animals performed significantly better than age-matched controls, whereas in other assays there were no differences. In addition to treatment effects, we also found several useful indicators of age-related decline in mice comparable to assays used to measure human frailty.

Email: fischerke@uthscsa.edu
Stress resistance and healthy aging in the naked mole-rat

Kaitlyn Lewis1,3 and Rochelle Buffenstein2,3

1Department of Cell & Structural Biology, University of Texas Health and Science Center, San Antonio, TX, USA; 2Department of Physiology, University of Texas Health and Science Center, San Antonio, TX, USA; 3Barshop Institute for Longevity and Aging Studies, University of Texas Health and Science Center, San Antonio, TX, USA

Resistance to toxins and other stressors has been shown to impact healthspan and longevity in multiple models of aging, including worms, flies, and rodents. The naked mole-rat, the longest-living rodent with a maximum lifespan of >31 years, demonstrates a profound resistance to a variety of toxins in vitro. Throughout this very long lifespan, naked mole-rats experience very little decline on physiological and biochemical levels with age, and cancer frequency is incredibly rare, with one case ever reported. We hypothesize that the nuclear factor-erythroid 2-related factor-2 [Nrf2] signaling cytoprotective pathway plays a larger role in this profound cytotoxicity and cancer resistance. Nrf2 is a transcription factor ubiquitously expressed in all tissues and conserved from worms to humans. Nrf2 degradation is regulated by kelch-like ECH-associated protein 1 [Keap1] that targets Nrf2 for ubiquitination and subsequent degradation via the proteasome. After a stressful insult to the cell (i.e. toxin, ROS), interactions between Nrf2 and Keap1 are inhibited and free molecules of Nrf2 are able to move into the nucleus to bind to the antioxidant response element (ARE) to activate the transcription of over 600 cytoprotective molecules, including those involved in detoxification, glutathione metabolism, heat shock factors, as well as subunits of the proteasome. Largely studied with regard to cancer, Nrf2 has also been shown to interact with p53 and p21, playing a role in modulation of the cell cycle and cancer progression. We find that naked mole-rats, in addition to other naturally long-living species, have constitutively elevated levels of Nrf2 signaling in addition to a profound resistance to toxins and carcinogens in vivo demonstrating smaller changes in body temperature, rapid Nrf2 signaling, and very few signs of acute damage compared to shorter-lived rodents. Nrf2 upregulation may be a highly conserved mechanism that contributes to prolonged healthspan and longevity.

Email: LewisKN@uthscsa.edu
Advanced paternal age: age-related changes in AP endonuclease I abundance in spermatogenic cells

Jamila R. Momand, Kristine S. Vogel, Rebecca A. Garcia, Kim E. Hildreth and Christi A. Walter

Department of Cellular & Structural Biology, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA

A 30% increase in the number of older fathers during the last three decades has made the paternal age affect an increasingly important topic in aging reproductive health. Using lacI transgenic C57Bl/6 male mice, in conjunction with mice harboring inactivated alleles of various base excision repair genes, we have determined that normal base excision repair activity is essential in maintaining a low mutant frequency in spermatogenic cells. Comparisons of base excision repair activity in nuclear extracts prepared from spermatogenic cells obtained from young, middle-aged, and old mice revealed that repair activity is reduced by 50% in extracts prepared from old mice. This decreased base excision repair activity appears to be mediated by reduced abundance of a key base excision repair protein, AP endonuclease I (APEN1). Our objective is to delineate the mechanisms mediating reduced base excision repair in spermatogenic cells with increasing age by identifying the molecular changes that result in reduced APEN1 abundance and activity. TRP53 has previously been shown to play a role in regulating APEN1 expression within the liver, but this role has yet to be identified in spermatogenesis or with regard to aging. In pachytene spermatocytes obtained from older aged animals, there is an 88 and 80% increase in the phosphorylation of Serine 15 and Serine 20, respectively, on TRP53. Both of these phosphorylation signals are required to activate TRP53 and induce its regulatory function within the cell. Immunohistochemistry data demonstrate that there is reduced APEN1 staining within the pachytene spermatocyte population, suggesting a possible role for TRP53 in male reproductive aging. However, Northern blot analysis revealed that the abundance of the Apex1 transcript remains constant with increasing age. This suggests that the age-related change in APEN1 abundance is regulated at the translational or post-translational level. Spermatogenic cells isolated from Apex1 heterozygous mice, also carrying the lacI mutation reporter, demonstrated an accelerated increase in germ cell mutagenesis. Conversely, transgenic mice that overexpress APEN1 were protected from the age-related increase in mutant frequency. Combined, these results indicate a strong relationship between APEN1 abundance and germline mutagenesis and suggest that TRP53 may be a regulator of APEN1 abundance in spermatogenic cells with increasing age.

Email: Momand@uthscsa.edu

A 30% increase in the number of older fathers during the last three decades has made the paternal age affect an increasingly important topic in aging reproductive health. Using lacI transgenic C57Bl/6 male mice, in conjunction with mice harboring inactivated alleles of various base excision repair genes, we have determined that normal base excision repair activity is essential in maintaining a low mutant frequency in spermatogenic cells. Comparisons of base excision repair activity in nuclear extracts prepared from spermatogenic cells obtained from young, middle-aged, and old mice revealed that repair activity is reduced by 50% in extracts prepared from old mice. This decreased base excision repair activity appears to be mediated by reduced abundance of a key base excision repair protein, AP endonuclease I (APEN1). Our objective is to delineate the mechanisms mediating reduced base excision repair in spermatogenic cells with increasing age by identifying the molecular changes that result in reduced APEN1 abundance and activity. TRP53 has previously been shown to play a role in regulating APEN1 expression within the liver, but this role has yet to be identified in spermatogenesis or with regard to aging. In pachytene spermatocytes obtained from older aged animals, there is an 88 and 80% increase in the phosphorylation of Serine 15 and Serine 20, respectively, on TRP53. Both of these phosphorylation signals are required to activate TRP53 and induce its regulatory function within the cell. Immunohistochemistry data demonstrate that there is reduced APEN1 staining within the pachytene spermatocyte population, suggesting a possible role for TRP53 in male reproductive aging. However, Northern blot analysis revealed that the abundance of the Apex1 transcript remains constant with increasing age. This suggests that the age-related change in APEN1 abundance is regulated at the translational or post-translational level. Spermatogenic cells isolated from Apex1 heterozygous mice, also carrying the lacI mutation reporter, demonstrated an accelerated increase in germ cell mutagenesis. Conversely, transgenic mice that overexpress APEN1 were protected from the age-related increase in mutant frequency. Combined, these results indicate a strong relationship between APEN1 abundance and germline mutagenesis and suggest that TRP53 may be a regulator of APEN1 abundance in spermatogenic cells with increasing age.

Email: Momand@uthscsa.edu
A method to find lifespan-associated point mutations in the mammalian proteome

Jeremy Semeiks

Biophysics and Medical Scientist Training Programs, University of Texas Southwestern, Dallas, TX, USA

Evolutionary theory predicts that selection pressure declines with age. On the molecular level, one consequence of this may be that proteins needed for long life show loss-of-function point mutations in short-lived species. We used multiple regression, controlling for both body mass and shared ancestry, to find these lifespan-associated point mutations in several thousand high-quality protein alignments from 33 mammalian species. Overall, we found many more mutations in our data than would be expected by chance. We present examples of proteins that may be relevant to mammalian aging.

Email: jeremy.semeiks@utsw.edu
Health span assessment of C57BL/6 mice

Lauren B. Sloane, Vanessa Y. Soto, Michael E. Walsh, Kavitha Sataranatarajan, Kathleen Fischer, Balakuntalem Kasinath, Holly Van Remmen, Arlan Richardson, and Steven N. Austad

1Barshop Institute for Longevity and Aging Studies, University of Texas Health Science Center at San Antonio, San Antonio, Texas, USA; 2Department of Cellular and Structural Biology, University of Texas Health Science Center at San Antonio, San Antonio, Texas, USA; 3Division of Nephrology, Department of Medicine, University of Texas Health Science Center at San Antonio, San Antonio, Texas, USA; 4Department of Physiology, University of Texas Health Science Center at San Antonio, San Antonio, Texas, USA; 5Research Service, Audie Murphy VA Hospital, South Texas Veterans Health Care System, San Antonio, TX, USA

Measures of health span offer a more unified index of overall aging than measuring lifespan or longevity alone, as health span includes assays on a range of functional systems within the individual. The objectives of this study were to identify robust and reproducible functional measures of healthspan and to determine the interrelationship of these measures, and perhaps measures predictive of mortality. The study uses 16 different assays (e.g. body composition, metabolic rate, gait analysis, nerve conduction, kidney function) to evaluate overall health span in C57BL/6 male mice of four different ages (4, 20, 28, and 32 months of age) in a cross-sectional design. Many of these assays show significant consistent changes with age. By identifying age-related changes in these functional patterns, we can begin to develop biomarkers of aging that will help guide future directions of aging research in animal models and clinical work. For example, geriatric frailty involves the interrelationship of multiple variables – involuntary weight loss, exhaustion, lack of spontaneous activity, slow walking speed, and reduced grip strength. By measuring these variables in aging rodents, we can develop models that explore the physiology and etiology of such complex phenomena and provide critical insights into the mechanisms associated with age-related decline.

Email: sloane@uthscsa.edu
Is rapamycin a dietary restriction mimetic? A microarray analysis approach

Wilson Fok¹, Yiqiang Zhang²,³, Adam Salmon², Arunabh Bhattacharyya², Carolina Livi⁴, Alex Bokov²,⁵, Jon Gelfond⁶, Bill Wood, Yongqing Zhang⁶, Kevin Becker⁶, Walter Ward²,³, Arlan Richardson¹,²,⁷ and Viviana Perez¹,²

¹Department of Cellular and Structural Biology, The University of Texas Health Science Center at San Antonio, San Antonio, TX, USA; ²Barshop Institute for Longevity and Aging Studies, The University of Texas Health Science Center at San Antonio, San Antonio, TX, USA; ³Department of Physiology, The University of Texas Health Science Center at San Antonio, San Antonio, TX, USA; ⁴Department of Molecular Medicine, The University of Texas Health Science Center at San Antonio, San Antonio, TX, USA; ⁵Department of Epidemiology & Biostatistics, The University of Texas Health Science Center at San Antonio, San Antonio, TX, USA; ⁶National Institute on Aging, Baltimore, MD, USA; ⁷Research Service, Audie Murphy VA Hospital (STVHCS), San Antonio, TX, USA

Rapamycin (rapa) has recently been found to extend lifespan in Saccharomyces cerevisiae, Drosophila, and mice. Based on epistasis studies in invertebrates, it has been hypothesized that rapa is a dietary restriction (DR) mimetic. To test the hypothesis, we used male C57BL6 mice fed ad libitum (AL), DR mice fed with 60% of the ad libitum diet, rapa mice fed ad libitum supplemented with encapsulated rapa (14 ppm; the same dose used by the studies which showed that rapa increased the lifespan of mice) in the food, and DR+Rapa mice fed with 60% of the diet consumed by ad libitum mice supplemented with encapsulated rapa (14 ppm). Microarray analyses were conducted to compare the similarities or differences in genes expression and signaling pathways associated with DR or rapa interventions for 6 months, starting at 2 months of age.

Our previous data indicated that there were no differences in body composition, with exception in fat mass, in which only DR treatment had a significant decrease in fat mass, and both treatment showed significantly reduced phosphorylation of S6 (60%), which indicates that both treatments affected mTOR activity similarly. Also, both treatment increased levels of autophagy when measured by the ratio of LC3II/LC3I. From our microarray analysis of the liver, we observed that DR has a more dramatic effect on genes than rapa. Our principal component analysis, which provides a broad overview of changes, showed that there was no overlap between rapa and DR, but there were some shared overlap of components between AL and rapa and also between DR and DR+Rapa. The gene analysis showed that DR and DR+Rapa share a large overlap of genes, whereas overlap between DR and rapa is fewer. We found similar results in our pathway analysis as well. Our preliminary data indicate that although DR and rapa may have similar effects on mTOR activity and autophagy, there are still major differences at the gene and pathway level and that the differences observed among both treatments suggest that rapamycin is not a DR mimetic.

Acknowledgement
This work was supported by NIH Grant AG036613.

Email: fok@uthscsa.edu
Proteome stability as a mechanism of longevity in marine bivalves

Stephen Treaster¹,4, Keith Maslin¹,4, Iain Ridgway⁵, Asish Chaudhuri¹,3 and Steven Austad¹,2,4

¹Barshop Institute for Longevity and Aging Studies, The University of Texas Health Science Center at San Antonio, San Antonio, TX, USA; ²Department of Cellular and Structural Biology, The University of Texas Health Science Center at San Antonio, San Antonio, TX, USA; ³Department of Biochemistry, The University of Texas Health Science Center at San Antonio, San Antonio, TX, USA; ⁴Department of Molecular Medicine, The University of Texas Health Science Center at San Antonio, San Antonio, TX, USA; ⁵School of Ocean Sciences, Bangor University, Wales, UK

Maintenance of protein structure and function has been implicated in the aging process in a variety of model organisms. We are utilizing a range of bivalve mollusk species, with lifespans ranging from under a decade to over 500 years, in a comparative study to investigate the hypothesis that long life requires superior proteome stability. These ages can be individually determined by counting growth rings in the shell. This experimental system provides a unique opportunity to study closely related organisms with vastly disparate longevities and their relative proteome stabilities.

Specifically, we are testing their ability to maintain structure and function under various stressors. The apolar, fluorescent BisANS probe binds to hydrophobic regions of proteins exposed as they unfold, incorporation being directly related to compromised tertiary structure. Protein aggregation and preservation of GAPDH function under stress was also measured. Stressors included TBHP as an oxidative stress, and urea as an unfolding agent.

To date, we find that stress-induced protein unfolding decreases with increasing lifespan. This stability corroborates with superior GAPDH function and decreased protein aggregation under the same stressors. Taken together, these data support the hypothesis that proteome stability is a determinant of longevity.

Email: Treaster@uthscsa.edu