EXPLORING OPPORTUNITIES AND FEASIBILITY OF TRIALS ON EFFECTS OF INCREASING NAD+ LEVELS IN OLDER ADULTS

WORKSHOP PROGRAM

National Institute on Aging Workshop
Exploring Opportunities and Feasibility of Trials on Effects of Increasing NAD+ Levels in Older Adults
December 9, 2021
Register for the workshop

December 9, 2021 (All times Eastern Daylight Time)

Time	Topic	Presenter
10:00–10:05 am	Welcome and Introductions	NIA, participants
10:05–10:15 am	Charge to participants	

Session I: Aging and NAD+ Level

Time	Topic	Presenter
10:15–10:45 am	Effect of nicotinamide mononucleotide on metabolic health & frailty	David Sinclair Harvard University
10:45–11:15 am	The NAD Metabolome Promotes Resiliency to Conditions of Metabolic Stress and 99% of What You've Been Told About Sirtuins and Longevity is Wrong	Charles Brenner City of Hope National Medical Center
11:15–11:40 am	The NAD World 3.0: the importance of the inter-tissue communication in mammalian aging and longevity control	Shin-Ichiro Imai Washington University
11:40-12:00 pm	NAD supplementation in diseases with DNA repair deficiency	Vilhelm Bohr National Institute on Aging, NIH
12:00-12:20 pm	Novel neuroimaging approaches to measure in vivo NAD+ and NADH	Fei Du McLean Hospital
12:20-12:50pm	Break	

Session II: Therapeutic Targets for Boosting NAD+ in Aging

Time	Topic	Presenter
12:50-1:10 pm	NAD precursors cycle between the host and gut microbiome	Joseph Baur University of Pennsylvania
1:10–1:30 pm	CD38 as a therapeutic target	Eduardo Chini Mayo Clinic
1:30–1:50 pm	Normalization of NAD+ redox balance as a therapy for heart failure	Rong Tian University of Washington
1:50–2:10 pm	Mitochondrial nutrient sensing as a target for therapeutic intervention	Michael Zemel NuSirt Biopharma & Kinexum
2:10–2:40 pm	Break	

Session III: Clinical Experience Targeting NAD Therapeutically

Time	Topic	Presenter
2:40-3:00 pm	Clinical evidence for targeting NAD therapeutically. *Review of completed clinical trials for NAD+ boosting*	Eric Verdin Buck Institute for Research on Aging
3:00–3:20 pm	Safety and metabolism of long-term administration of nicotinamide riboside	Andrew Shao ChromaDex Inc.
3:20-3:40 pm	Oral Supplementation of NAD+ Precursors for Promoting Healthy Cardiovascular Aging	Douglas Seals University of Colorado
3:40–4:00 pm	Break	
4:00–4:20 pm	NAD+ boosting compounds to improve memory and cerebrovascular function	Christopher Martens University of Delaware
4:20–4:40 pm	Sirtuin-NAD activators in COVID-19 infection	Shalender Bhasin Brigham and Women's Hospital, Harvard University
4:40–5:00 pm	Effect of nicotinamide mononucleotide (NMN) on cardiometabolic function	Samuel Klein Washington University
5:00–5:45pm	What should we try to treat or prevent and who is the target population(s)? What types of trials are feasible and needed?	Group Discussion

Index of Speaker Biographies and Abstracts

Session I

David Sinclair, Ph.D., A.O., Harvard Medical School
Dr. Sinclair is a Professor in the Department of Genetics, Blavatnik Insitute, and co-Director of the Paul F. Glenn Center for Biology of Aging Research at Harvard Medical School. He is best known for his work on understanding why we age and how to slow its effects. He obtained his Ph.D. in Molecular Genetics at the University of New South Wales, Sydney, in 1995 and did his postdoctoral research at M.I.T. with Dr. Leonard Guarente where he co discovered a cause of aging for yeast as well as the role of Sir2 in epigenetic changes driven by genome instability and aging. In 1999 he moved to Harvard Medical School and has primarily focused on understanding why we age and the role of protective enzymes called the sirtuins, which respond to changing NAD+ levels, exercise, and caloric restriction (CR). The Sinclair lab was the first to identify a role for NAD biosynthesis in the regulation of lifespan and first showed that sirtuins are involved in CR's benefits in mammals and identified the first small molecules that activate SIRT1 (STACs). His lab is also working on epigenetic changes as a driver of aging and the use of reprogramming factors to reset the age of cells and tissues. He has published over 200 scientific papers, is a co-inventor on over 50 patents, and is the New York Times bestselling author of Lifespan (2019). He serves as co-chief editor of the scientific journal Aging and has received 35 honors including the Australian Medical Research Medal, the Irving Wright Award, the NIH Director's Pioneer award.

Effect of nicotinamide mononucleotide on metabolic health & frailty
Alice E Kane, Colleen Carmody, Michael Schultz, Joao Amorim, Xiao Tian, David A. Sinclair
Abstract: NAD is an essential co-factor for over 400 enzymatic reactions, as a regulator of protein-protein interactions, and as a co-substrate for enzymes involved in regulating DNA repair and aspects of aging, such as the PARPs and Sirtuins. Previous studies have demonstrated that raising NAD levels in old animals with declining NAD has a range of health benefits, including the restoration of DNA repair, increased angiogenesis and mitochondrial function in brain and muscle, and protection of the kidney from ischemic damage. Here we investigate the effect of chronic life-long administration of an NAD precursor, nicotinamide mononucleotide (NMN, 600mg/kg in drinking water), from 13 months of age in male and female C57BL/6 mice. Male control mice had increased body weight over their lifespan and this was significantly reduced in the NMN group, despite similar food intake. Dual energy X-ray absorptiometry (DEXA) showed that the NMN treated males, compared to controls, had greater lean mass and reduced fat mass at 24 months of age. Additionally, the NMN treated males were more active in the dark phase and showed better metabolic flexibility as demonstrated by lower and more flexible respiratory exchange ratios. FRIGHT (Frailty Inferred Geriatric Health Timeline) and AFRAID (Analysis of Frailty and Death) clock scores are novel machine-learning based predictors of age and lifespan built on frailty index data (http://frailtyclocks.sinclairlab.org/). At 23 months of age

NMN-treated mice had lower frailty scores and were predicted to live longer than untreated mice, consistent with preliminary data in small cohorts indicating that NMN-treated mice have longer median lifespans than untreated mice. Overall, this data provides preclinical evidence that chronic NMN treatment improves frailty and metabolic health in aging. An update on the human clinical trials with NAD boosters will be given. Funding: A.E.K is supported by an AFAR Irene Diamond postdoctoral award. D.A.S. is supported by the Paul F. Glenn Foundation for Medical Research, Edward Schulak, VoLo Foundation and NIH grants R01DK100263 and R37AG028730. DS is a board member and consultant to MetroBiotech, a company developing NAD boosters to treat diseases. DS outside activities are at sinclair.hms.harvard.edu/david-sinclairs-affiliations.

Charles Brenner, Ph.D., City of Hope National Medical Center
Charles Brenner is a leading biochemist and expert on NAD metabolism, who discovered the eukaryotic nicotinamide riboside kinase pathway, established quantitative targeted NAD metabolomics, and has described a wide variety of conditions of metabolic stress that disturb the NAD system transcriptionally and at the level of metabolites. Trained at Stanford (PhD, 1988-1993) and Brandeis (post-doc, 1993-1996), Dr. Brenner served on the faculty of Thomas Jefferson University (1996-2003), Dartmouth College (2003-2009), University of Iowa (head of Biochemistry, 2009-2020) prior to his recruitment to City of Hope to serve as the inaugural chair of their new department of Diabetes & Cancer Metabolism. The developer of valuable intellectual property in the NAD space, he also serves as chief scientific advisor of ChromaDex, consultant to Cytokinetics and Ridgeline, and co-founder of Alphina. His current research focuses on NAD metabolism in the context of women's health, virology, inflammation, cancer, fatty liver disease and rare human diseases.

The NAD Metabolome Promotes Resiliency to Conditions of Metabolic Stress and 99% of What You've Been Told About Sirtuins and Longevity is Wrong
Abstract: All living organisms have gene sets that delimit the conditions in which they are able to live, repair themselves and reproduce. Evolutionary biology has established that multiple parameters have been under selective pressure throughout animal evolution including resistance to pathogens, starvation and harsh conditions; and the capacities for early fertility, high fecundity and maximizing the numbers of cycles of reproduction. However, once offspring are born and afforded sufficient protection to permit their own reproductive success, parents are generally dispensable. Though animals evolved to successfully reproduce many times, they do not generally outlive their reproductive capacity and are under little selective pressure to do so. Thus, animals have gene sets that permit their brains, circulatory, muscular and digestive systems to last long enough to support their ability to produce offspring, whether their characteristic lifespans are measured in days or hundreds of years. The exception that proves the rule is the human female, who has evolved to outlive her reproductive capacity by roughly one generation time, largely because humans are highly dependent

on mothering to achieve reproductive success. This notion—that longevity has not been under direct selection in animal evolution but rather integrates fitness measures that support successful reproduction—has been tested in flies: only by selecting for old flies to mate for hundreds of generations was it possible to obtain longer lived flies. The resulting genetic changes were not monogenic. Against this powerful theoretical and experimental backdrop, it is surprising that molecular biologists so frequently propose monogenic longevity genes. Based on one model of aging in one yeast, SIR2 was proposed to be such a gene. However, the ability of an old mother S. cerevisiae to form a daughter operates at a population frequency of less than 1 per 2^{21} and is not conceivably under selective pressure; sir2-deleted cells are advantaged in the other model of yeast aging; and the mechanism by which SIR2 extends lifespan in yeast (suppressing formation of ribosomal DNA circles) is not conserved in other organisms. Nonetheless, it was claimed that extra copies of SIR2 homologs extend lifespan in worms and flies, which led to a global cavalcade of exploration of SIR2-related genes, termed sirtuins, in which phenotypes were characterized in terms of longevity. Long after mice transgenic for SIRT1 failed to extend lifespan, the collaborative efforts of researchers from 9 institutions showed that the worm and fly results were strain-specific and not driven by SIRT1 homologs. Billions of dollars were lost to so-called sirtuin-activating compounds that did not convincingly bind to their specific targets—targets which fail to act as mediators of longevity. Today, interest has shifted to boosting the NAD metabolome, which I argue has little to do with regulation of sirtuins, much to do with maintenance of electron flow and repair processes, and is challenged by common and disease-associated conditions of metabolic stress, potentially more than aging itself. NAD-related trials will benefit from assessment of repair and inflammation informed by transcriptomic and metabolomic analysis of the NAD system. In contrast, the globally influential edifice of sirtuins as longevity genes can be recognized as intellectually bankrupt, posing an ongoing massive opportunity cost to biomedical progress and meaningful public health information.

Shin-ichiro Imai, M.D., Ph.D., Washington University in St. Louis

Shin-ichiro Imai received his MD and PhD degrees in 1989 and 1995, respectively, from Keio University School of Medicine in Tokyo, Japan. From 1987 to 1997, he studied cellular aging-associated transcriptional regulation in human fibroblasts and proposed his "Heterochromatin Island Hypothesis of Aging." In 1997, he moved to the US and joined the laboratory of Leonard Guarente at the Massachusetts Institute of Technology as a Human Frontier Science Program Long-Term Fellow. There, he made a paradigm-shifting discovery of the NAD+-dependent protein deacetylase activity of yeast and mammalian Sir2 proteins and published his landmark paper in the journal *Nature* in 2000. In 2001, he joined the faculty of Washington University School of Medicine in St. Louis, Missouri, and is currently Professor in the Departments of Developmental Biology and Medicine. Professor Imai's laboratory has been studying the systemic regulatory mechanisms of mammalian aging and longevity control, focusing on roles of mammalian sirtuins and NAMPT-mediated NAD^+ biosynthesis in mammals. Based on his research,

he proposed a novel concept of a systemic regulatory network for mammalian aging/longevity control, named the "NAD World" in 2009. This concept has been evolving to "NAD World 2.0" in 2016 and now to "NAD World 3.0."His long-term goal is to achieve "productive aging," which aims to make our later lives as healthy and productive as possible, by understanding the spatial and temporal dynamics of the NAD World and developing nicotinamide mononucleotide (NMN), a critical NAD^+ intermediate, as an effective anti-aging intervention for humans. Since 2017, Professor Imai has also been serving as the Invited Chief Scientist and leading a research group in the Institute for Biomedical Research and Innovation in Kobe, Japan. He has received many prestigious awards for his works, including the American Society for Cell Biology/Glenn Foundation Award, the Ellison Medical Foundation New Scholar Award in Aging, the American Diabetes Association Innovation Award, the Juvenile Diabetes Research Foundation Innovation Award, the Glenn Award for Research in Biological Mechanisms of Aging, the WUSM 2008 Distinguished Investigator Award, the Ellison Medical Foundation Senior Scholar in Aging Award, Glenn/AFAR Breakthroughs in Gerontology (BIG) Award, and International Okamoto Award. He was also selected as one of "The Most Influential 100 people for Japan 2017" by Nikkei Business. He is living with his wife in the suburb of St. Louis and enjoying his Midwest life.

The NAD World 3.0: the importance of the inter-tissue communication in mammalian aging and longevity control

Our major interest is to understand the systemic regulation of aging and longevity in mammals and translate that knowledge into an effective anti-aging intervention that could make our later lives as healthy and productive as possible ("productive aging"). Our previous studies have identified three key tissues as critical elements in mammalian aging and longevity control: the hypothalamus as the control center, skeletal muscle as an effector, and adipose tissue as a modulator. The inter-tissue communication between these tissues plays an important role in counteracting age-associated functional decline and determining healthspan and lifespan. In particular, we have demonstrated that adipose tissue communicates with the hypothalamus by secreting extracellular nicotinamide phosphoribosyltransferase (eNAMPT), the rate-limiting NAD+ biosynthetic enzyme in mammals, via extracellular vesicles (EVs) and counteract age-associated functional decline and promote longevity in mice. EV-contained eNAMPT secreted from adipose tissue has an important role in maintaining NAD+ biosynthesis and the activity of SIRT1, the mammalian NAD+-dependent protein deacetylase, in the hypothalamus. Indeed, supplementing eNAMPT-containing EVs purified from young mice significantly increases hypothalamic NAD+ levels, improves wheel-running activity, and extends lifespan in aged mice. We have recently identified a new neuronal subpopulation in the hypothalamus, particularly in the dorsomedial hypothalamic nucleus (DMH). These neurons regulate white adipose tissue function through the sympathetic nervous system specifically directed to white adipose tissue and increase the secretion of eNAMPT-containing EVs, impacting the process of aging. Now that it has become a consensus that systemic decline in NAD+

availability is a critical driving force for age-associated functional decline, boosting NAD+ levels in key tissues by eNAMPT-containing EVs could be another effective anti-aging intervention, in addition to the use of nicotinamide mononucleotide (NMN), a product of the NAMPT enzymatic reaction and a key NAD+ intermediate. In my presentation, I will present the NAD World 3.0, a comprehensive concept explaining these multi-layered inter-tissue communications for mammalian aging and longevity control, and further discuss our efforts of developing preventive/therapeutic anti-aging interventions by using eNAMPT-containing EVs and NMN to achieve "productive aging" for the sake of our rapidly aging society.

Vilhelm Bohr, M.D., Ph.D., National Institute on Aging, NIH

My early professional training took place at the University of Copenhagen, Denmark, where I earned an M.D. in1978, and both Ph.D. and D.Sc. degrees in 1987. After training in neurology and infectious diseases at the University Hospital in Copenhagen, I undertook postdoctoral studies in Biochemistry in the laboratory of Dr. Hans Klenow at the University of Copenhagen, where I first became interested in nucleic acid metabolism. I developed this interest further when I held a Visiting Scholar position in the laboratory of Dr. Philip Hanawalt at Stanford University from 1982-1986. In 1986, I obtained a Junior Investigator appointment at the National Cancer Institute (NCI), and advanced to a tenured Senior Investigator appointment in 1988. In 1992, I was appointed Chief of the Laboratory of Molecular Genetics at the National Institute on Aging (NIA). Throughout my career, I have made significant contributions and advanced understanding of DNA repair pathways and mechanisms and the cellular response to oxidative DNA damage and oxidative stress. I have also been especially interested in the repair and function of the mitochondrial genome. Early in my career, I developed a widely used method for studying DNA repair in the transcribed portion of the genome and found that transcriptionally-active genes are preferentially repaired through a process now known as transcription-coupled nucleotide excision repair (TC-NER). The discovery of TC-NER provided strong evidence of the tight interaction between the cellular machineries for DNA repair and transcription in mammalian cells. In my recent studies, I have made seminal findings about the relationships between DNA damage, DNA repair capacity and aging-associated neurodegeneration, and have proposed important models describing crosstalk between the nuclear and mitochondrial genomes, as well as the importance of energy homeostasis/imbalance and mitochondrial dysfunction in aging-related neurodegenerative disease. Most, but not all of the research in my laboratory is funded through the intramural research program at NIA.

NAD supplementation in diseases with DNA repair deficiency

Abstract: We find that some DNA repair defective diseases with severe neurodegeneration have mitochondrial dysfunction. Our studies involve cell lines, the worm (c.elegans), and mouse models and include the premature aging syndromes Xeroderma pigmentosum group A, Cockaynes syndrome, Ataxia telangiectasia and Werner syndrome. It also includes models of Alzheimers Disease. We find a pattern of

hyperparylation, deficiency in the NAD+ and Sirtuin signaling and mitochondrial stress, deficient mitophagy. We are pursuing mechanistic studies of this signaling and interventions at different steps to improve mitochondrial health and neurodegeneration. NAD supplementation stimulates mitochondrial functions including mitophagy and stimulates DNA repair pathways. Based on human postmortem material and IPSC cells we identify mitophagy defects as a prominent feature in Alzheimers disease (AD). Using c.elegans AD models we screened for mitophagy stimulators and identified compounds that subsequentially also show major improvement of AD features in mouse models. Cockayne syndrome mice have hearing deficit that reflects the one seen in the patients. Short term NAD supplementation eliminates the hearing loss. The hearing loss is a of a similar type seen in age related hearing loss. Treatment of mice with NR improves the age related hearing loss. Our studies suggest that NAD supplementation can have great benefit under conditions where is depleted such as DNA repair defects. Clinical trials are ongoing or planned.

Fei Du, Ph.D., McLean Hospital

Dr. Fei Du is an Associate Professor of Psychiatry in Harvard Medical School, director of Laboratory for High-Field Imaging and Translational Neuroscience at McLean Hospital. He is a neuroimaging physicist with a broad background in Magnetic Resonance Imaging and Spectroscopy (MRI/MRS) technical developments. After the rigorous training in technical aspects of MRI/MRS, he has begun to use his expertise in neurobiology research on neuropsychiatric disorders since joining McLean Hospital. His research interests evolve to combine MRI/MRS technique developments with "translational imaging". The goal is to outline the trajectories of biological abnormalities and to identify treatment-engaged targets, exploring biomarkers of disease progression and treatment response in neuropsychiatric disorders.

Novel neuroimaging approaches to measure in vivo NAD+ and NADH

Abstract: Mitochondrial dysfunction and cellular energy deficits have emerged as hallmarks of aging and age-associated disorders, such as Alzheimer's disease (AD). Cellular metabolism and mitochondrial function are mediated, in part, by the oxidized and reduced form of nicotinamide adenine dinucleotide (i.e. NAD+ and NADH, respectively). Unfortunately, decreases in NAD+, and consequently in the redox ratio (RR=NAD+/NADH)—i.e., the balance between oxidized and reduced forms of NAD—are associated with numerous diseases, including AD. Importantly, animal and cell-line studies suggest NAD+ supplementation can slow or reverse these age-related abnormalities. For example, nicotinamide riboside (NR)—an over the counter orally bioavailable precursor to NAD+ that enhances mitochondrial function—has been shown to elevate the RR and reduce neuroinflammation, improve cerebrovascular function, and reduce amyloid aggregation in the preclinical animal models. Therefore, in vivo probes are needed, which could provide crucial information about NAD-related bioenergetic abnormalities in AD and, in turn, facilitate the continued development and refinement of this promising treatment approach. However, measuring NAD+/NADH in vivo is challenging due to extremely low concentrations (<1 mM) and overlapping

resonances with other metabolites. To that end, we have developed/implemented novel techniques (31P-MRS with 1H decoupling and magnetization transfer) to measure NAD+/NADH and other markers of mitochondrial function with functional ties to NAD, including activity of creatine kinase, ATPase, and glutathione—a molecule essential for cellular repair. These advances may provide the biomarkers, which aim to document and quantify the neurobiological and clinical effects of NAD supplementation in AD using in vivo brain imaging techniques.

Section II

Joseph Baur, Ph.D., University of Pennsylvania

Joseph Baur is an Associate Professor in the Department of Physiology and the Institute for Diabetes, Obesity, and Metabolism at the Perelman School of Medicine of the University of Pennsylvania. He has made key contributions to the understanding of how metabolism and dietary factors influence longevity. In 2006, Dr. Baur and colleagues showed that a sirtuin activator, resveratrol, is able to improve insulin sensitivity and ameliorate premature mortality in obese mice. He led a team that revealed a mechanism accounting for off-target effects of rapamycin, a drug that extends life in mice, but has side effects that limit its utility in humans. His laboratory at Penn is currently focused on the use of small molecules to understand and mimic the health-promoting effects of caloric restriction in rodents, with a particular focus on nicotinamide adenine dinucleotide metabolism. The Baur lab employs genetic models, isotopic labeling strategies, and metabolomics to probe the pathways by which NAD is synthesized and influences whole-body metabolism. These methods are also employed to study the effects of supplemental NAD precursors and to explore compartmentalization of NAD pools. Recently, the Baur lab co-discovered the first transporter that facilitates entry of NAD into mammalian mitochondria, where it can directly influence fuel selection and metabolic capacity. Dr. Baur has co-authored more than one hundred peer-reviewed publications, as well as several book chapters and numerous invited commentaries and reviews.

NAD precursors cycle between the host and gut microbiome

Abstract: Nicotinamide adenine dinucleotide (NAD) is a redox cofactor essential to all living organisms, including microbes that reside in the gut. It is widely perceived that microbes residing in the gut lumen utilize dietary precursors to synthesize NAD. We used isotopically labeled precursors to demonstrate that dietary precursors such as tryptophan, aspartate, and nicotinic acid are absorbed in the proximal part of the gastrointestinal tract and not available to microbes residing in the distal parts of the gut. Surprisingly, we find that host-derived nicotinamide originating in the circulation enters the gut lumen and serves as a precursor for microbial NAD synthesis. In the lumen of the small intestine, this pathway accounts for most NAD synthesis, whereas in the colon NAD production appears to be about equally split between host-derived nicotinamide and de novo synthesis from sugars and amino acids generated from dietary fiber. In turn, host tissues utilize the nicotinic acid generated by microbes for

NAD synthesis, providing metabolic flexibility and maintaining circulating nicotinic acid levels even in the absence of dietary nicotinamide or nicotinic acid. We further demonstrate that microbial metabolism of oral nicotinamide riboside in mice can result in therapeutically relevant concentrations of circulating niacin, which has not previously been appreciated. Thus, NAD precursors cycle between the host and gut microbiome in a mutually beneficial manner to maintain NAD homeostasis.

Eduardo Chini, M.D., Ph.D., Mayo Clinic

Dr. Chini is a clinician-investigator at Mayo Clinic. He has been at Mayo for 28 years. His laboratory at the Kogod Center on Aging (Minnesota) and Department of Anesthesiology- (Florida) studies metabolism and metabolic reprograming in aging and age-related diseases. In particular, Dr, Chini's laboratory has been the pioneer on CD38 biology its role of NAD metabolism and also in its pre-clinical developments. Dr. Chini's laboratory has been funded over the years by NIH, AFAR, Glenn laboratories, Helen Diller Foundation, FAER, industry, and by the Mayo Foundation.

CD38 as a therapeutic target

Abstract: Aging is the main risk factor for several diseases and is one of the main risk factors for significant morbidity and mortality. Aging is characterized by the development of metabolic dysfunction and loss of metabolic flexibility. These include dysregulation in energy homeostasis and decline in tissue levels of Nicotinamide Adenine Dinucleotide (NAD). These changes appear to be at least in part responsible for the decline in healthspan and resilience observed in aging. However, to date if age-related NAD-decline can indeed lead to molecular and physiological changes observed during the aging process is not known. Thus, several questions such as can NAD-decline in vivo lead to the development of molecular hallmarks of aging such as cellular senescence, telomer and autophagy dysfunction have not been approached experimentally. However, even if NAD-decline is not a driving force of molecular aspects of aging it Is now clear that tissue and cellular NAD-decline is implicated in several pre-clinical animal models of human diseases

Rong Tian, M.D., Ph.D., University of Washington

Dr. Tian is professor and director of the interdisciplinary Mitochondria & Metabolism Center at the University of Washington. Her work is recognized in three inter-related areas of cardiovascular diseases: bioenergetics, metabolism, and mitochondrial biology. In the past twenty years, her laboratory has made seminal contributions to the field by combining a vigorous in vivo metabolic phenotyping with the powerful technology of multi-nuclear NMR spectroscopy, proteomics and metabolomics. Dr. Tian's recent work on NAD metabolism, cellular stress response and inflammation has yielded a major stimulus to the translational research as heart failure becomes a predominant diagnosis in our aging and obese population. Dr. Tian received numerous award and honors including Distinguished Achievement Award of the American Heart Association (AHA) Basic Science Council, Research Achievement Award of the International Society for

Heart Research (ISHR) and 2021 George E Brown Lecturer of the AHA. She is currently the Editor in Chief for Journal of Molecular and Cellular Cardiology.

Normalization of NAD+ Redox as a Therapy for Heart Failure

Abstract: Recent studies suggest that changes in intracellular NAD+ level or NAD(H) redox state in the heart are linked to mitochondrial dysfunction and progression of heart failure. The dietary supplements NAD+ precursors have been shown to improve mitochondrial function in preclinical models of heart failure with either reduced or preserved ejection fraction. However, mechanisms of action underlying the benefits of boosting NAD level are not fully understood. Targeting NAD metabolism holds promise but is at early stage in the development as clinical therapies for heart failure. Further Studies are needed to determine the cause of altered NAD metabolism and to identify the patient population that likely benefits from the therapy.

Michael Zemel, Ph.D., NuSirt Biopharma & Kinexum

Michael Zemel received his PhD in Physiology and Nutritional Biochemistry at the University of Wisconsin-Madison. He then served on the faculties of Endocrinology and of Nutrition at Wayne State University and as Research Endocrinologist at the VA Medical Center associated with Wayne State from 1980 – 1990, where his work focused on endocrine regulation of cell signaling and downstream effects on both blood pressure regulation and insulin sensitivity as well as clinical trials of novel cardiometabolic therapeutics. He moved to the University of Tennessee in 1990 where he served as Professor of Nutrition and of Medicine and expanded his work to focus on energy sensing, muscle-fat cross-talk and regulation of adipocyte metabolism and development and evaluation of obesity therapeutics. Michael founded NuSirt in 2007 and left the university in 2012 as Professor Emeritus to devote his full attention to the company, where he led research and drug discovery programs focused on energy-sensing and health-span, including therapeutics for metabolic diseases associated with aging and over-nutrition. He now serves as Chief Scientific Officer Kinexum, Inc. where he assists other companies in translational drug product development and subsequent clinical development.

Nutrient Sensing: Enhancing NAD^+ Precursor Activity

Abstract: NAD^+ replenishment via precursors, such as nicotinamide riboside (NR) and nicotinamide mononucleotide (NMN) has elicited promising results in extending lifespan and healthspan and treating age-related cardiometabolic and neurodegenerative diseases in preclinical models. However, multiple clinical studies of NR have not demonstrated efficacy, while a recent small study of NMN demonstrated a modest but significant effect on muscle insulin sensitivity in older women. NAD^+ replenishment exerts multiple effects, one of which is as a substrate for sirtuins; it is possible that NAD^+ concentration achieved via supplementation of NR or NMN is not sufficient to provide a clinically meaningful level of sirtuin activation. We have shown leucine is an allosteric activator of both Sirt1 and Sirt3, reducing the Km for NAD^+ of both by 50-75% and have demonstrated that combining leucine with sirtuin pathway

activators produces significant improvements in weight, insulin sensitivity, lipids and blood pressure in clinical trials. We have also shown that leucine synergizes with both NR and NMN to increase sirtuin activity and increase lipid oxidation in adipocytes, hepatocytes and skeletal muscle cells by 50-100%. Further, concentrations of NR and NMN that caused only modest (25%) increases in median lifespan in *C. elegans* synergized with leucine to increase lifespan by 100% and 225% for NR- and NMN-leucine combinations, respectively. Leucine also synergized with NR in LDL-receptor KO mice, significantly reducing circulating lipids and regressing atherosclerotic lesion size and macrophage infiltration at concentrations that exerted no independent effects. Thus, combining NAD^+ precursors with leucine holds promise to enhance sirtuin-mediated effects on healthspan-related outcomes.

Section III

Eric Verdin, M.D., Buck Institute for Research on Aging

Dr. Eric Verdin is the President and Chief Executive Officer of the Buck Institute for Research on Aging. A native of Belgium, Dr. Verdin received his Doctorate of Medicine from the University of Liege and completed additional clinical and research training at Harvard Medical School. He has held faculty positions at the University of Brussels, the National Institutes of Health (NIH), and the Gladstone Institute at the University of California San Francisco (UCSF). Dr. Verdin is currently adjunct Professor of Medicine at UCSF and Adjunct Professor at the University of Southern California (USC). Dr. Verdin studies how metabolism, diet, and small molecules impact epigenetic regulatory mechanisms, and thereby the aging process and its associated diseases. He is a highly cited scientist (top 1 percent) and has been recognized for his research with multiple awards including a fellowship from the American Association for the Advancement of Science, election to the American Society for Clinical Investigation, the Association of American Physicians, and Belgium's Royal Academy of Medicine. The Buck Institute, in the San Francisco Bay Area, is globally recognized as the pioneer and leader in the field of research on aging, the number one risk factor for chronic disease. For more information visit www.buckinstitute.org

Clinical evidence for targeting NAD therapeutically. Review of completed clinical trials for NAD+ boosting

Abstract: Nicotinamide adenine dinucleotide (NAD) metabolism is altered during aging with important consequences in terms of intermediary metabolism and other key regulators of the aging process such as sirtuins and PARPs. New interventions based on replenishing the depleted NAD stores have emerged based on NAD precursors such as NR and NMN. Despite several decades of active investigation and numerous possible biochemical mechanisms of action suggested, only a small number of randomized and adequately powered clinical trials of NAD precursors as a therapeutic strategy have taken place. I will review the existing literature in this field and discuss possible challenges and opportunities created by these new interventions.

Andrew Shao, Ph.D., ChromaDex, Inc.
Dr. Andrew Shao has spent over two decades in the global nutrition industry, assuming leadership roles in various nutrition, scientific, regulatory and government affairs functions. He currently serves as Sr VP, Global Scientific & Regulatory Affairs for ChromaDex Corp where he oversees the company's scientific, regulatory, R&D and quality functions. Prior to joining ChromaDex Dr. Shao held several leadership positions at Herbalife Nutrition, and served as Sr. VP Scientific & Regulatory Affairs for the Washington, DC-based trade association, the Council for Responsible Nutrition (CRN). Before joining CRN, he was a senior scientist at General Nutrition Corporation (GNC), and previously, in research and development at Kemin. Dr. Shao has advised governments around the world on science-based regulatory and policy reform on topics ranging from health claims, to risk analysis to regulation of botanicals. He is the author or co-author of over 60 peer-reviewed articles, abstracts, trade articles and book chapters and serves on the Editorial Board of several peer-reviewed journals. He is the current Chair of the Sustaining Partners Roundtable of the American Society for Nutrition, and member of the Tufts Nutrition Council. Dr. Shao holds a doctorate in nutritional biochemistry and master's in human nutrition science, both from Tufts University, and a bachelor's in biology from Brandeis University.

Safety and metabolism of long-term administration of nicotinamide riboside
Abstract: Nicotinamide riboside (NR) supplementation has been studied in a total of 24 published clinical trials to date, either alone or in combination with other active ingredients, mostly in adults, including various patient groups. Most studies have utilized the form of NR that has also achieved successful regulatory notification and/or approval (NRCl) and tested doses up to 2000 mg/day for up to 12 weeks and 1000 mg/day for up to 5 months. Safety-related outcome measures include standard blood chemistry, liver function and spontaneously reported adverse events. Metabolism of NR in humans has been assessed through measurement of the NAD metabolome ("NADome") or select NAD metabolites in biological fluids and tissues in 11 studies. One published study examined NR supplementation in a group of patients that included children, and while there are no published studies on NR in pregnant/lactating women, no specific safety concern has been identified in these groups. Given the role of methyl donors in the metabolism and excretion of NAD precursors, the effect of NAD precursor supplementation on the methyl donor pool, and the resulting physiologic consequences, if any, remain to be addressed. Similarly, the effect of NR and other NAD precursor supplementation on cancer outcomes and how these are impacted by dose and duration of supplementation requires further exploration. Overall, results from published clinical studies demonstrate that NR supplementation is well tolerated in adults, with no attributable adverse effects.

Douglas Seals, Ph.D., University of Colorado
Doug Seals is a Distinguished Professor of Integrative Physiology at the University of Colorado Boulder. Professor Seals obtained M.S. and Ph.D. degrees in applied

physiology at the University of Wisconsin-Madison and performed postdoctoral research training in applied physiology and aging in the Department of Medicine at Washington University School of Medicine in St. Louis under the late Professor John Holloszy. The primary research interest of his laboratory is in establishing evidence-based strategies for promoting healthy cardiovascular aging. Professor Seals has published >350 peer-reviewed journal articles, has trained over 250 undergraduate and graduate students, postdoctoral fellows, and junior faculty, and has served as principal investigator on numerous grant awards from the National Institutes of Health, including a MERIT award from the National Institute on Aging.

Oral Supplementation of NAD+ Precursors for Promoting Healthy Cardiovascular Aging

Abstract: Scientific premise. Cardiovascular diseases (CVD) remain the leading cause of death in developed societies and advancing age is the primary risk factor for CVD. As the number of older adults continues to increase worldwide, marked increases in CVD burden are expected in the absence of novel, evidence-based interventions. Increases in systolic blood pressure (SBP), vascular endothelial dysfunction, and stiffening of the large elastic arteries, particularly the aorta, are major antecedents and independent predictors of age-related CVD risk. Excessive vascular superoxide bioactivity (oxidative stress) resulting in reduced bioavailability of the vasodilatory and vascular-protective molecule nitric oxide (NO) and adverse structural changes in the arterial wall including increased collagen deposition and elastin degradation, are key mechanisms driving increased SBP and vascular dysfunction with aging. Reductions in the bioavailability of the ubiquitous co-enzyme and enzymatic co-substrate nicotinamide adenine dinucleotide (NAD+) with aging is associated with increased superoxide production and oxidative stress.

Working hypothesis. We hypothesized that dietary supplementation with natural precursors aimed at increasing NAD+ bioavailability would lower SBP and improve vascular function with aging.

Research and results. In a proof-of-concept study, we showed that oral supplementation with the NAD+ precursor nicotinamide mononucleotide (NMN) (drinking water) restored NO bioavailability and endothelial function in old mice to levels observed in young adult controls and reduced age-related aortic stiffness by normalizing vascular superoxide bioactivity, reducing oxidative stress, decreasing collagen, and preserving elastin in the arterial wall (de Picciotto et al, 2016 https://pubmed.ncbi.nlm.nih.gov/26970090/). To translate these observations to humans, we first performed a small pilot clinical trial in which the NAD+ precursor nicotinamide riboside (NR; 500 mg 2x/day) or placebo control were administered for 6 weeks each using a randomized, double-blind, crossover study design in adults 55-79 years of age (n=24) without serious clinical disorders (ClinicalTrials.gov NCT02921659). NR was safe and well-tolerated and reduced SBP and aortic stiffness (carotid-femoral pulse wave velocity), particularly in individuals with above-normal baseline SBP (>120 mmHg) (Martens et al., 2018 https://pubmed.ncbi.nlm.nih.gov/29599478/). Based on

these findings, we are presently conducting a larger NIA-funded (R01 AG061514) randomized clinical trial of NR (500 mg 2x/day) with a more clinically relevant (3-month) treatment duration using a double-blind, parallel group study design (ClinicalTrials.gov NCT03821623) in adults >50 years of age with above-normal SBP (n=120; 60/group; 30F/group).
Conclusions. Overall, our work is providing a rigorous, broadly translational experimental approach and growing foundation of evidence for establishing the safety and efficacy of natural NAD+ precursor compounds for promoting healthy CV aging.

Christopher Martens, Ph.D., University of Delaware
Dr. Martens is an Assistant Professor of Kinesiology & Applied Physiology at the University of Delaware and Director of the Neurovascular Aging Laboratory. He completed postdoctoral training at the University of Colorado Boulder where he was among the first to demonstrate that chronic nicotinamide riboside (NR) supplementation raises blood-cellular NAD+ concentrations in humans. His current research seeks to understand how cardiometabolic risk factors contribute to impaired brain blood flow and risk for late-life cognitive impairment in humans. In this regard, he is currently funded by the National Institute on Aging (NIA) to investigate the efficacy of NR supplementation for improving memory and cerebrovascular function in older adults with amnestic mild cognitive impairment (MCI), a prodromal form of Alzheimer's disease.

NAD+ boosting compounds to improve memory and cerebrovascular function
Abstract: Aging is associated with an accumulation of multiple cardiometabolic risk factors that contribute to cognitive impairment risk of Alzheimer's disease and other forms of dementia. NAD has recently been linked to the development of vascular and neuronal dysfunction and is therefore a compelling target for the prevention of age-related cognitive impairment. The purpose of this presentation is to discuss the potential of NAD-boosting compounds for treating or preventing age-related cognitive impairment, primarily through the targeting of cardiometabolic risk factors. The aims and endpoints of an ongoing clinical trial will be shared along with design considerations for future trials of NAD-boosting compounds.

Shalender Bhasin, M.D., Brigham and Women's Hospital, Harvard University
Dr. Shalender Bhasin is a Professor of Medicine at the Harvard Medical School, and Director of the Research Program for Men's Health and Aging at the Brigham and Women's Hospital in Boston, MA. He is also the Director of the Boston Claude D. Pepper Aging Research Center. Dr. Bhasin is an internationally recognized expert in Men's Health and aging. He has published more than 400 original research papers in top tier journals, has led some of the most important randomized trials of the benefits and risks of testosterone and other function promoting therapies in older adults, elucidated the mechanisms of testosterone action, and clarified the role of circulating hormone binding proteins. Dr. Bhasin has been the recipient of numerous teaching and research awards. He was the recipient of the Outstanding Clinical Investigator Award

from the Endocrine Society and Frontiers in Science Award from American Association of Clinical Endocrinologists.

Nicotinamide Adenine Dinucleotide (NAD) Augmentation to Attenuate the Severity and Improve Outcomes in Older Adults with SARS-CoV-2 Infection

Abstract: A majority of people infected with the SARS-CoV-2 remain asymptomatic or suffer from only a mild respiratory illness; however, a subset of patients develop a more severe illness that can progress rapidly to acute respiratory distress syndrome, multi-organ failure, and high risk of death. An unbridled, heightened inflammatory response and the excessive release of cytokines in critically ill patients with COVID-19 pneumonia contributes to the development of acute respiratory distress syndrome and multi-organ failure, similar to that observed in severe cases of other major respiratory viral diseases, such as severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS), respiratory syncytial virus, and influenza. The recognition of the important role of aggressive inflammatory response in the pathophysiology of ARDS and multiorgan failure in severe COVID-19 disease has led to the hypothesis that immunomodulatory therapy that can attenuate the inflammatory response could modulate disease severity and improve outcomes.
Nicotinamide adenine dinucleotide (NAD) serves as a co-factor in signaling pathways that regulate innate immunity to respiratory viruses (e.g., the influenza, SARS, MERS, and SARS-CoV-2), inflammation, and cell survival. Older people have low levels of NAD, and SARS-CoV-2 infection further depletes NAD levels by upregulating CD38 and poly-ADP-ribose polymerases (PARPs). Older individuals have lower levels of NAD and are at increased risk for COVID-19 infection, developing a more severe disease, and of dying from the disease. Raising cellular NAD levels by administration of its precursor β nicotinamide mononucleotide, NMN) boosts innate immunity to other coronaviruses and be beneficial in improving outcomes in patients with COVID-19, particularly in patients with at risk co-morbidities which heighten inflammation and also increase susceptibility to negative outcomes of COVID-19. We have shown that oral administration of a crystalline formulation of β nicotinamide mononucleotide substantially raises the intracellular NAD levels. Randomized clinical trials to determine the efficacy of NAD augmentation in reducing the severity of SARS-CoV-2 infection and reduce the length of hospital stay are in progress.

Samuel Klein, M.D., Washington University

Samuel Klein M.D. is the William H. Danforth Professor of Medicine, Director of the Center for Human Nutrition, Director of the Center for Applied Research Sciences, Chief of the Division of Geriatrics and Nutritional Sciences, and Director of the Weight Management Program at Washington University School of Medicine in St. Louis, Missouri. Dr. Klein received an MD degree from Temple University Medical School, and an MS Degree in Nutritional Biochemistry and Metabolism from the Massachusetts Institute of Technology. He completed residency training in Internal Medicine and a Clinical Nutrition fellowship at Boston University Hospital, a Nutrition and Metabolism

Research fellowship at Harvard Medical School, and a Gastroenterology fellowship at The Mt. Sinai Medical Center in New York. He is board certified in Internal Medicine, Gastroenterology, and Nutrition. Dr. Klein is past-president of the North American Association for the Study of Obesity and the American Society for Clinical Nutrition, and inaugural chair of the Integrative Physiology of Obesity and Diabetes NIH study section. He was elected to the American Society for Clinical Investigation in 1996 and to the American Association of Physicians in 2008. Dr. Klein has had consistent R01 funding from the NIH since 1990, and has published more than 450 papers in nutrition, metabolism, and obesity. He has received numerous awards for his research, including the American Gastroenterological Association (AGA) Miles and Shirley Fiterman Foundation Award in Nutrition, the AGA Masters Award for Outstanding Achievement in Basic or Clinical Research in Digestive Sciences, the AGA Obesity, Metabolism & Nutrition Research Mentor Award, the Academy of Science-St. Louis Award for Outstanding Achievement in Science, the American Society for Nutrition Robert H. Herman Award, the American Society for Parenteral and Enteral Nutrition George Blackburn Research Mentorship Award, and The Obesity Society TOPS Research Achievement Award, George A. Bray Founders Award, and the George L. Blackburn Award for Excellence in Obesity Medicine, the American Society for Parenteral and Enteral Nutrition George Blackburn Research Mentorship Award, and the Gerald M. Reaven Distinguished Leader in Insulin Resistance Award. Dr. Klein's research activities are focused on understanding the mechanisms responsible for metabolic dysfunction associated with obesity, particularly nonalcoholic fatty liver disease, and the therapeutic effects of weight loss. Dr. Klein is also committed to training young investigators and clinicians in nutrition and obesity and has provided mentorship to 48 trainees in clinical and translational metabolic research.

Effect of nicotinamide mononucleotide (NMN) on cardiometabolic function

Studies in rodents have shown obesity and aging impair tissue nicotinamide adenine dinucleotide (NAD+) biosynthesis, which contributes to metabolic dysfunction. The availability of nicotinamide mononucleotide (NMN) is an important rate-limiting factor in mammalian NAD+ biosynthesis. We conducted a 10-week, randomized, placebo-controlled, double-blind trial to evaluate the effect of NMN supplementation on metabolic function in 25 postmenopausal women with prediabetes who were overweight/obese. Insulin-stimulated glucose disposal, assessed by using the hyperinsulinemic-euglycemic-clamp procedure, increased by 25±7% ($P<0.01$) in the NMN group, which was accompanied by an increase in insulin-stimulated phosphorylated AKT in skeletal muscle ($P<0.01$), whereas neither outcome changed after placebo treatment. Body composition (fat mass, fat-free mass, intra-abdominal fat, intrahepatic triglyceride content) and muscle mitochondrial respiratory capacity did not change after treatment with placebo or NMN. These results demonstrate NMN improves muscle insulin sensitivity in women with prediabetes who are overweight/obese, independent of changes in body composition or mitochondrial function.