OSU Oregon State University

The Linus Pauling Institute

FALL-WINTER 2015 **RESEARCH NEWSLETTER**

From the Director

Balz Frei, Ph.D.

LPI Director and Endowed Chair
Distinguished Professor of Biochemistry and Biophysics
Joan H. Facey LPI Professor

I have decided to step down as LPI director and retire from Oregon State University at the end of June 2016. By then, I will have served as director of the Linus Pauling Institute for 19 years—a very long time for any director of a center or institute. I have had a very productive and highly rewarding time here at LPI and OSU, and it has been a true privilege and pleasure to build the Institute from its modest beginnings in Weniger Hall to where we are today in the state-of-the-art Linus Pauling Science Center. I am proud of our new home in LPSC; all the excellent faculty, students, and staff I have recruited to LPI; and the top-notch institute we have built together.

When I joined LPI in 1997, I was the only principal investigator in the Institute; we now have 12 PIs working in three major areas of research: Healthy Aging, Cardiometabolic Disease Prevention, and Cancer Prevention and Intervention. Together, we have published 933 scientific publications over the past 18 years, which have been cited by our peers in their publications over 26,000 times—impressive numbers that attest to our prolific scholarly output and the significant impact of our work.

The most highly cited publication from LPI, with almost 800 citations, is a review article on "Tea catechins and polyphenols: Health effects, metabolism, and antioxidant functions," co-authored by the late Dr. Jane Higdon and myself. Other highly cited articles focus on the antioxidant functions and health benefits of vitamins C and E; the roles of oxidative stress and inflammation in aging and neurodegeneration, in particular Lou Gehrig's disease; the mechanisms by which lipoic acid enhances energy metabolism and reverses many of the age-related declines in mitochondrial and cellular functions; the mechanisms and role of phytochemicals (plant chemicals that may

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Diet and Optimum Health Conference

Stephen Lawson
Administrative Officer

LPI convened our eighth Diet and Optimum Health Conference, co-sponsored by the Oxygen Club of California, on the OSU campus in Corvallis from September 9th to 12th. The conference featured 37 speakers from around the world and was organized into three main sessions:

- *Vitamin E: A Critical Nutrient for Development and Health*
- *Extending Healthspan: Basic Concepts, Underlying Mechanisms, and the Role of Diet and Lifestyle*
- *Celebrating the Life and Work of George Bailey*

Sixty posters depicting experimental projects were displayed beginning Thursday evening. Ten of these were selected for consideration for Young Investigator Awards. Additionally, ten submitted abstracts were chosen for oral presentations.

Saturday morning featured a public session with presentations by two LPI Principal Investigators. **Balz Frei** discussed "LPI's Rx for Health—or how to meet your micronutrient needs," while **Tory Hagen** talked about "Adding life to your years and years to your life." Videos of their presentations can be viewed at http://bit.ly/1LiTnwt.

The Linus Pauling Institute Prize for Health Research was presented posthumously on Friday evening to **George Bailey** of Oregon State University for his seminal work in toxicology and carcinogenesis.

Vitamin E: A Critical Nutrient for Development and Health, *chaired by* ***Maret Traber*** *(LPI)*

- **Maret Traber** (LPI) discussed her latest research on the role of vitamin E (alpha-tocopherol) in zebrafish brain

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affect health) found in cruciferous vegetables in preventing colorectal and other cancers; and how vitamin D enhances immune function and protects against infectious diseases—to name just a few.

Our research has been supported by gifts from donors like many of you, as well as funding from Oregon State University, industry, the National Institutes of Health, and other federal agencies. Over the past 18 years, LPI researchers have brought over $50 million from NIH and some other extramural research grants and contracts to the Institute. In addition, thanks mainly to gifts from planned giving and bequests from our most generous supporters, LPI's endowment has more than quadrupled since the Institute's inception at OSU.

One of the accomplishments I am most proud of is our Micronutrient Information Center—an online resource of scientifically accurate and peer-reviewed information regarding the roles of vitamins, minerals, phytochemicals, and other dietary factors, including some food and beverages, in preventing disease and promoting health. This website has become our public face and is very popular among our peers, health professionals, the media, and the public, with over 1.5 million users annually from around the world. It has helped the Institute become one of the most trusted sources of balanced, evidence-based nutrition information in the world.

I am also fond of the Healthy Youth Program (HYP) in the Institute. It is our way to serve the local community and instill healthy diet and lifestyle habits into school children and their families. As we know, it takes a village to raise a child, and the HYP does an excellent job working with local schools, health authorities, and community organizations to teach children life skills that will put them on the right health trajectory for the rest of their lives. By disseminating their successful programs through peer-reviewed publications, online instruction manuals, and social media, the HYP does its part to help stem the obesity epidemic in this country.

The University's Research Office will initiate a national search very soon for a new director, and in my final column of the LPI Newsletter next Spring, I will give you an update on the search. Thank you for your advocacy and support of me personally and of our research and outreach programs in the Linus Pauling Institute over the past 19 years—we couldn't have done it without you! I think Linus Pauling would be proud. LPI

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development. Embryos from adult zebrafish fed low dietary levels of alpha-tocopherol exhibit brain and eye malformations, and fish die five days after birth. This implies that vitamin E sufficiency may be very important for the developing fetus in pregnant women even before they know that they're pregnant. Mechanistically, it appears that vitamin E protects an omega-3 fatty acid (docosahexaenoic acid, DHA) in the brain from peroxidation and depletion. Low levels of vitamin E also deplete choline in the brain. DHA and choline play important roles in neurodevelopment and in the health of adult brains. Many Americans do not consume the RDA for vitamin E (22.5 IU/day); food fortification may be one tactic to improve this.

• Many people in South Asia suffer from multiple micronutrient deficiencies (vitamin A, folate, iron, zinc, etc.), resulting in increased infections, impaired growth and cognitive development, and an increased risk for death in children and adults. **Keith West** (Johns Hopkins University) noted that over 50% of children and women of reproductive age in Nepal and Bangladesh are vitamin E deficient, although the situation may be improving as more vegetable oils—a good source of vitamin E—are consumed. In Bangladesh, pregnant women with vitamin E deficiency have a risk for miscarriage about twice that of women not deficient.

• **Chandan Sen** (The Ohio State University) presented results from his studies on the protection against stroke damage by low levels of tocotrienol, a member of the vitamin E family found in oat bran, nuts, wheat, palm oil, and corn. However, alpha-tocopherol did not protect against stroke-induced neuronal damage in animal models. Tocotrienol has long-lasting effects because it is stored in fat tissue and released over time. It works by several mechanisms, including the support of blood vessel formation, inhibition of pathways of neurodegeneration, induction of neuronal survival factors, and enhanced blood flow to stroke-damaged areas. A large clinical trial to test the effect of tocotrienol on patients at high risk for stroke because they had already had a small stroke began in 2013.

• About one-third of Americans are afflicted with metabolic syndrome, a condition that predisposes to diabetes and heart disease. Weight-loss programs to address this syndrome have largely failed because long-term compliance is poor. **Richard Bruno** (The Ohio State University) discussed how metabolic syndrome lowers vitamin E status. Metabolic syndrome is associated with inflammation and oxidative stress that decrease intestinal absorption and impair liver metabolism of vitamin E, suggesting that vitamin E requirements may be higher for people with metabolic syndrome. Improved vitamin E status may, in turn, protect against the fatty liver disease nonalcoholic steatohepatitis (NASH), which often occurs with metabolic syndrome. In related studies, it was found that the amount of dairy fat consumed with vitamin E had little effect on its absorption.

• **Mark Levine** (National Institutes of Health) talked about the cellular transport of ascorbic acid (vitamin C) and dehydroascorbic acid (DHA, the oxidation product of ascorbic acid). Ascorbic acid uses two specific transporters to enter most cells, whereas DHA uses glucose (sugar) transporters. Mouse experiments revealed that vitamin C gets into red blood cells as DHA, using the glucose transporters, and then gets reduced to ascorbic acid. Red blood cells deficient in vitamin C and/or vitamin E become very fragile and rigid and break apart (hemolysis). Since glucose competes with DHA for entry into red blood cells, diabetics may have impaired vitamin C status in these cells, leading to oxidative stress, hemolysis, and hypoxia (lack of oxygen) in the microvasculature. Additionally, in diabetics low levels of vitamin C in red blood cells may cause the destruction of vitamin E in those cells by oxidants, leading to hemolysis.

Extending Healthspan: Basic Concepts, Underlying Mechanisms, and Role of Diet and Lifestyle: Session 1

Risk Factors that Limit Healthspan,

chaired by ***Tory Hagen*** *(LPI) and* ***Viviana Pérez*** *(LPI)*

• While aging is a major risk factor for chronic disease, most attention has been focused on specific diseases rather than understanding the basic biology of aging. **Felipe Sierra** (National Institutes of Health) urged a new research emphasis on healthspan (geroscience) rather than disease. Extending lifespan will delay the onset of morbidities, and extending healthspan will compress those morbidities into a shorter period. There are problems with using standard rodent models to study disease because they are homogeneous and don't truly mimic human disease. Older people often have co-morbidities from multiple diseases or conditions that aren't represented in animal models. Also, some cancer immunotherapies that work in young mice are lethal in old mice. Research has shown that physiological aging is malleable, unlike chronological aging.

• **Mohammad Forouzanfar** (University of Washington) discussed the Global Burden of Diseases, Injuries, and Risk Factors study, a global effort involving millions of subjects and over 1,000 scientists in 188 countries that began in 1990. Dietary intake of the subjects was assessed every five years, and data on disease incidence, disability, and mortality were collected and analyzed. Deaths attributable to poor diet increased during the study period due to population growth and aging. Poor diet (high intakes of salt, processed meat, and sugar) reduced life expectancy and healthspan, particularly in Central Asia, East Asia, and Central and Eastern Europe. Reducing the intake of salt, processed meat, and sugar and increasing the consumption of fruit, vegetables, and nuts are the recommended interventions.

• Some animal species live very long lives: the naked mole rat has a lifespan of about 30 years compared to about three years for the ordinary rat, and certain species of bats and mollusks are especially long lived. **Steven Austad** (University of Alabama) suggested that these animals may provide insight into extending healthspan in people. Long-lived animals share an ability to very effectively maintain protein homeostasis—the resistance to protein oxidation and aggregation and the means to efficiently get rid of misfolded and damaged proteins. Understanding the mechanisms underlying protein homeostasis may help us to develop dietary strategies to retard senescence. Ordinary lab mice are not good models to study healthspan because they differ so much from wild mice, which are leaner, eat more sparingly, and consume food with nutrient densities different from the chow fed to lab mice. Several long-term studies on caloric restriction in primates to extend lifespan may have had conflicting results because of significant differences in the basal diets.

Inflammation, Redox Biology, and Healthy Aging,

chaired by ***Adrian Gombart*** *(LPI) and* ***Kathy Magnusson*** *(LPI)*

• **Miriam Capri** (University of Bologna) addressed the age-related problem of "inflamm-aging," which is characterized by sustained low-level inflammation, chronic activation of certain immune cells called macrophages, oxidative damage, and a physiological blurring of "self" and "non-self." Inflammation is associated with many age-related diseases like atherosclerosis, diabetes, and cancer (for more details, see http://bit.ly/1HVpG9Z). While nuclear and mitochondrial DNA affect longevity, the gut microbiome also plays a role. The gut microbiome declines in diversity with age and, in centenarians, exhibits altered proportions of bacterial phyla. There are over 100 bacterial phyla in the gut and over 1,000 species; the biological function of about half of these is still unknown. Inflammation also occurs in centenarians, but these long-lived people show resilient adaption that increases healthspan.

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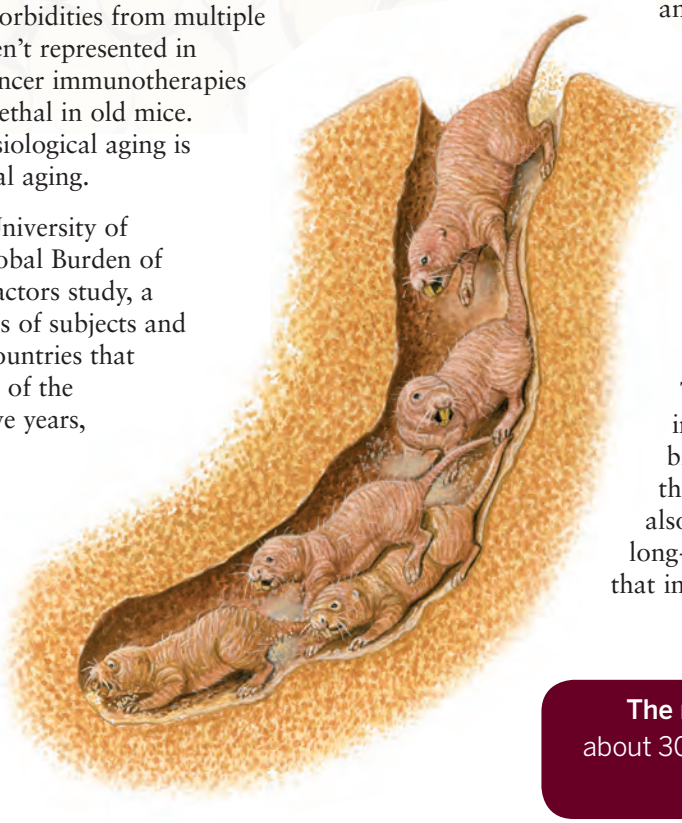

The naked mole rat has a lifespan of about 30 years compared to about three years for the ordinary rat.

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• Senescent cells have stopped dividing, but they continue to be metabolically active and secrete pro-inflammatory molecules that help in wound healing and injury repair but are otherwise damaging. Their accumulation in organs and tissues is associated with age-related diseases. **Darren Baker** (Mayo Clinic) explained that smoking, excessive alcohol intake, high-fat diets, and other factors induce cellular senescence. Vitamin E and other antioxidants, as well as exercise, may be protective. In specially bred mice, the genetic removal of senescent cells delays the onset of age-related diseases like cancer and increases lifespan.

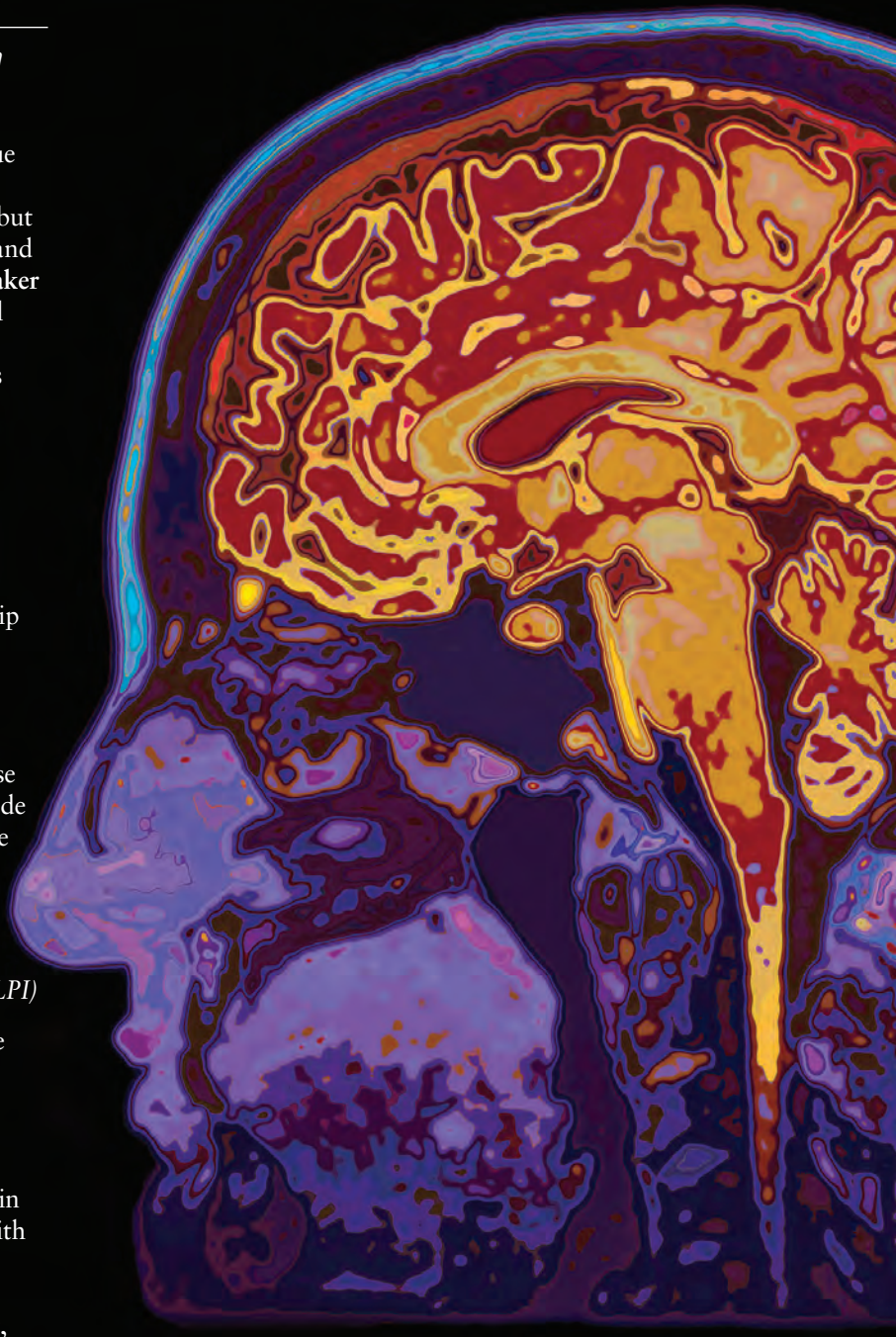
• **Dean Jones** (Emory University) and Helmut Sies proposed the "redox code"—a set of principles that underlie the metabolism of oxygen and the way that organisms interact with their environment. The relationship between the cumulative exposure to environmental factors—called the exposome—and the genome largely predicts health and disease. Oxygen is central to the interface between the exposome and genome. Animals evolved in an oxygen-rich environment, and their cells use oxygen and reactive oxygen species like hydrogen peroxide as signaling molecules. Aging is associated with a decline in the adaptability of the "exposure memory systems" that govern the response to the environment.

Strategies to Maintain Cognitive Function,

chaired by Kathy Magnusson (LPI) and Adrian Gombart (LPI)

• **Kathy Magnusson** (LPI) discussed the cognitive decline that accompanies aging: worsening memory, attention, cognitive flexibility, and executive function (decision-making, organizing goal-oriented behavior, and prioritization). World and verbal knowledge, as well as short-term memory and language, are largely preserved in older age. Rodents share similar declines in cognition with people, so they are good models for research. The aging brain retains some plasticity—studies have shown that exercise, an enriched environment, and mental training, as well as caloric restriction, dietary polyphenols, and polyunsaturated fats, can improve cognitive and motor functions like balance and gait instability in older age. Although the number of neurons doesn't decline much with age, they shrink and the number of synapses decreases. Exercise increases neurogenesis and brain volume, an enriched environment increases neurogenesis, vitamin D increases certain brain receptors, lipoic acid improves long-term memory, and a Mediterranean diet that emphasizes polyunsaturated fats and flavonoids helps old brains.

• While long-term caloric restriction has been shown in several animal species to extend lifespan, its more important effect may be in extending healthspan by attenuating age-related cognitive decline and delaying the onset of chronic diseases. Because meaningful caloric restriction must be severe (20-50% reduction in calories) and long term to



Vitamin D increases certain brain receptors, lipoic acid improves long-term memory, and a Mediterranean diet that emphasizes polyunsaturated fats and flavonoids helps old brains.

manifest benefits, investigators have searched for substances that mimic caloric restriction without problems of compliance. **Donald Ingram** (Louisiana State University) discussed some of these compounds, including resveratrol (found in peanuts, grapes, and wine) and rapamycin (a naturally occurring antibiotic found in specific soil). Caloric restriction mimetics typically affect glucose metabolism, insulin sensitivity, and/or autophagy, the process by which cells digest and recycle damaged molecules. Many caloric restriction mimetics also improve motor function and cognition in older animals. Proof of efficacy in people is very difficult to obtain.

• **Andrew Scholey** (Swinburne University of Technology, Australia) focused on the role of nutriceuticals, such as theanine from tea, curcumin from the spice turmeric, and polyphenols from cocoa, in maintaining good cognitive function throughout life. In clinical trials, curcumin improved working memory and attention and decreased fatigue after four weeks of supplementation. Cocoa polyphenols improved cognition, blood flow, and neurogenesis in the brain. Theanine enhanced task-relevant cognitive functions, and broad micronutrient supplements improved cognition, mood, sleep, energy, and alertness, possibly by correcting insufficiencies. Many of these functions can now be studied by neuroimaging techniques that elucidate effects on the brain vasculature and neuronal networks.

• Risk factors for cardiovascular disease (CVD) change with age, but age itself is not sufficient to describe these changes. **Michelle Odden** (Oregon State University) discussed these relationships, with an emphasis on the impact of frailty, which is the loss of physiological reserve and increased vulnerability to stress, assessed by gait speed, fatigue, and strength. A body mass index (BMI) greater than 35 is associated with an increased risk for CVD in adults younger than 60. Conversely, in adults over 70, a low BMI is associated with a higher risk for CVD. In adults aged 65 to 84, a low systolic blood pressure is associated with lower risk for CVD, whereas a higher systolic blood pressure is protective in adults 85 and older. Low blood pressure in the very old and frail may predispose to falls and injuries, and high blood pressure may compensate for increased arterial stiffness. Optimal blood pressure varies according to frailty. Reduced kidney function is also linked to a higher risk for mortality in octogenarians.

• **Lynn Marshall** (Oregon Health & Science University) talked about risk factors for falls and bone fractures. Low-impact falls (from standing height or less) are the primary reason for emergency room visits in older adults. Falls often result in fractures of the hip, wrist, or spine, with a higher rate among women. Osteoporosis and low vitamin D status increase the risk for fractures from falls. Falls are also the major cause of death from injury. Hip fractures cause enormous disability and result in high rates of mortality. Vertebral fractures cause chronic, disabling pain and are associated with a two-fold increase in the risk for death. Falls are associated with polypharmacy (patients taking multiple drugs) and impaired vision, cognition, and physical function, as well as back pain and urinary urge incontinence at night. Walking for exercise helps lower the risk for falls. A large Swedish study found that older adults that consumed less than three servings of fruit and vegetables per day had an increased risk for fall-related hip fractures.

• **Sean Newsom** (Oregon State University) addressed the impact of exercise on insulin resistance, which is common among the 90 million Americans that are pre-diabetic. Obesity and diabetes are closely linked—lipid accumulation impairs insulin action. Exercise protects against diabetes and improves glucose metabolism and insulin sensitivity, but the effect is short-lived. A ten-day period without exercise increases blood glucose, but dramatic improvements in insulin sensitivity lasting up to three days can be seen after one exercise session, regardless of age. Weight loss and exercise lower the incidence of diabetes in men and women.

Beginning in 1979, Bailey developed trout as a low-cost model with high statistical power for studies in carcinogenesis.

Celebrating the Life and Work of George Bailey,

chaired by ***Rod Dashwood*** *(Texas A&M University) and* ***Tom Kensler*** *(University of Pittsburgh)*

• George Bailey, who died in October 2014, had a seminal research career at Oregon State University, where he retired as a Principal Investigator in LPI (http://bit.ly/1S00JdQ). **Richard Scanlan** (Oregon State University) gave a biographical sketch of Bailey's career, which began at OSU in the Sinnhuber Aquatic Research Laboratory (SARL). SARL gained widespread recognition in the 1960s due to

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Oxygen Club of California World Congress


Valencia, Spain

The Oxygen Club of California held its annual world congress, "Oxidants and Antioxidants in Biology," in Valencia, Spain, at the end of June. The large meeting featured 45 presentations on nutrients and medicine, inflammation, aging and longevity, vascular disease, and neurodegenerative diseases. Some highlights are summarized below.

• **Bharat Aggarwal** (University of Texas M. D. Anderson Cancer Center, Houston) discussed the role of spice-derived phytochemicals, especially curcumin from turmeric (found in curry), in cancer prevention. Cancer is associated with chronic inflammation and the activation of a DNA transcription factor called NF-κB, resulting in cell proliferation, blood vessel formation, and metastases. In cell culture studies, curcumin inhibits NF-κB through the production of reactive oxygen species; this inhibition is abrogated by cellular antioxidants like glutathione.

• Procyanidins are large molecules in cocoa, apples, grapes, and peanuts that, although not absorbed by intestinal cells, are associated with decreased risk for colorectal and gastric cancers. **Patricia Oteiza** (University of California-Davis) talked about the ways that procyanidins exert beneficial biological effects. "Lipid rafts" in cell membranes control many processes, including cell signaling and "tight junctions" between cells. Procyanidins prevent the disruption of lipid membranes and help maintain tight junctions between cells, thereby limiting the inappropriate exposure to certain molecules. The interactions between procyanidins and cell membranes is complex and partly depends on the type of lipids in the membranes. Procyanidins are metabolized by gut bacteria into smaller compounds that can be absorbed into the blood stream and exert important biological effects.

• Lipoic acid has many beneficial effects in cell cultures and rodents: it inhibits inflammatory molecules in endothelial cells that line blood vessels and arteries, decreases lipid synthesis, inhibits atherosclerosis, lowers body weight and triglycerides, and acts as an antioxidant. **Balz Frei** (Linus Pauling Institute) presented the results of a recent study designed to assess the role of lipoic acid on inflammation, oxidative stress, and body weight in overweight and obese people. Lipoic acid (600 mg/day) or placebo was given to 64 men and women for 24 weeks. Lipoic acid decreased biomarkers of lipid oxidation and inflammation and, in the very obese and in overweight or obese women, lowered body weight.

• Aggregated or damaged cellular proteins affect cell health. Several speakers, including **Tilman Grune** (German Institute of Human Nutrition, Potsdam) and **Bertrand Friguet** (Sorbonne Universités, Paris), addressed the role of aggregated or damaged proteins in aging and disease. Oxidized proteins can aggregate and inhibit the cell's proteasomes, which are structures that digest and rid cells of damaged proteins. Such inhibition may contribute to the development of Alzheimer's disease. Oxidized proteins also impair glucose metabolism in cells, contributing to cellular senescence.

• **Leocadio Rodriguez-Mañas** (Getafe University Hospital, Spain) discussed the problems of frailty and sarcopenia in older adults. Frailty refers to the increased vulnerability and decreased physiological reserve of strength in older age. Frailty is associated with inflammation, oxidative stress, and other dysfunctions that predispose to disability. An important deficit of frailty is sarcopenia, which is characterized by a decrease in muscle mass and an increase in fat tissue. In sarcopenia, mitochondria, the organelles in cells that produce energy, decline in number and function. An effective treatment for frailty involves aerobic exercise and resistance and strength training (twice a week for 30-45 minutes) and good nutrition.

• **Giuseppe Poli** (University of Turin, Italy) addressed the role of oxysterols in brain disease. Oxysterols are oxidation products of cholesterol that, unlike cholesterol itself, freely cross the blood-brain barrier. In neuronal cell cultures, oxysterols have been shown to induce the synthesis of beta-amyloid, which accumulates in the brains of Alzheimer's patients. Chemical modifications of a protein called Tau are associated with increasing severity of Alzheimer's disease. Two main types of oxysterols (24OH and 27OH) have conflicting effects on Tau, but the speaker speculated that the accumulation of 27OH in the brain may contribute to disease progression.

• Soy isoflavones have been studied for a possible role in cancer prevention, although the evidence to date is inconclusive. **José Viña** (University of Valencia, Spain) reported remarkable effects of one soy isoflavone, genistein, fed to mice used as models for Alzheimer's disease. The amount of beta-amyloid, a hallmark of Alzheimer's disease, in the brain was significantly reduced, and that correlated with improved cognition, including learning, memory, and odor discrimination. Clinical trials are needed to confirm this therapeutic approach in humans.

The 2015 Health Sciences Prize was given to **Giuseppe Poli** for his extensive and seminal work on oxidized lipids, such as hydroxynonenal and oxysterols, and their role in human diseases like atherosclerosis, liver fibrosis, inflammatory bowel disease, and Alzheimer's disease.

Additionally, a number of awards, including one sponsored by the Linus Pauling Institute, were made to young investigators. LPI

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the discovery by Sinnhuber that aflatoxin in moldy cottonseed oil fed to hatchery trout caused liver cancer. Beginning in 1979, Bailey developed trout as a low-cost model with high statistical power for studies in carcinogenesis. Bailey was also renowned as a wine aficionado and for his interest in playing bluegrass music, fly fishing, surfing, and restoring old trucks.

• **Roger Coulombe** (Utah State University) discussed the attributes of the trout model to study carcinogenesis. Trout have a low rate of spontaneous tumors, sensitivity to many carcinogens, and, after exposure to carcinogens, develop tumors caused by gene mutations relevant to human tumors. Trout are also very economical, which, as mentioned above, allows the use of thousands of animals to achieve high statistical power. For example, Bailey used 42,000 trout to determine the effect of very low-dose exposures to dibenzo[a,l]pyrene, a ubiquitous environmental contaminant released by the burning of carbon fuels—similar to real-world exposures in people.

• Glucobrassicin is a compound found in cruciferous vegetables like broccoli, cabbage, and Brussels sprouts. When these vegetables are chopped or chewed, an enzyme converts glucobrassicin into indole-3-carbinol (I3C), which has been shown in trout studies to inhibit liver cancer caused by exposure to aflatoxin. In the stomach, I3C is converted into diindolylmethane (DIM). **David Stresser** (Corning, Inc.) noted that the protective mechanisms of DIM are still unknown but may involve cell cycle arrest and apoptosis (programmed cell death), the disruption of cell signaling, the induction of enzymes that detoxify carcinogens, and the inhibition of enzymes that activate carcinogens. Surprisingly, the timing of the dose is important—when I3C is given after the initiation of cancer by a carcinogen, it may actually promote carcinogenesis. Therefore, supplementation with I3C may have risks.

• **Ashok Reddy** (Oregon Health & Science University) discussed the implications of Bailey's large trout studies on risk assessment in people. Typically, human hazards are estimated by extrapolating the high doses of carcinogens used in rodent studies to the low doses that people encounter in the environment. However, this process has a lot of unavoidable uncertainty, especially since rodents have a significant incidence of spontaneous tumors. Trout studies showed a linear relationship between liver tumors and low-dose aflatoxin exposure, unlike the non-linear response to low doses of the widespread environmental carcinogen dibenzo[a,l]pyrene. While these studies cannot prove a threshold level of exposure, they are useful to predict a practical threshold and should help the EPA develop reasonable guidelines.

• **Rod Dashwood** (Texas A&M University) was a post-doctoral fellow in Bailey's lab who later worked with him on chlorophyll studies. Chlorophyll, the green pigment in plants, had previously been shown to be antimutagenic, but new studies demonstrated its anticancer action. Chlorophyll binds to aflatoxin, which causes liver cancer, and polycyclic aromatic hydrocarbons in the gut, forming complexes too big to be absorbed into the blood stream and reach target organs like the liver. Indole-3-carbinol from cruciferous vegetables can block liver cancer caused by aflatoxin exposure by the same mechanism. Freeze-dried spinach, a terrific source of chlorophyll, inhibited colon tumors in rats treated with heterocyclic amines, or "cooked-meat mutagens," formed from the high-temperature cooking of proteinaceous food.

• Once experiments had established that chlorophyll and its derivatives could prevent liver cancer in trout and rodents exposed to aflatoxin, Bailey was anxious to find out if people would respond the same way. He and **Thomas Kensler** (University of Pittsburgh) conducted a clinical trial in Qidong, a region in China where people are unavoidably exposed to aflatoxin in their food and, consequently, have a very high incidence of liver cancer. The participants were

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given either a placebo or 100 mg of chlorophyllin (a stable compound derived from chlorophyll) three times a day just before each meal for four months. After three months, urine was collected and examined for markers of DNA damage that reasonably predict cancer. The level of such markers in the urine of people who got the chlorophyllin was 55% less than those getting placebo, suggesting that their cancer risk is lower. Current trials in China utilize broccoli preparations containing glucoraphanin and sulforaphane to evaluate their effect on the detoxification of air pollutants.

• **Dave Williams** (LPI) was Bailey's colleague at OSU for many years. They worked together to establish the pharmacokinetics (absorption, metabolism, and excretion) of aflatoxin and to develop a method to extract chlorophyll from spinach at low cost. About 95% of the human exposure to carcinogenic polycyclic aromatic hydrocarbons (PAHs) comes from food. Recent studies have used accelerator mass spectrometry to establish the pharmacokinetics of a PAH called dibenzo[def,p]-chrysene that will enable the assessment of very low-dose exposure to PAHs and possible micronutrient or dietary interventions.

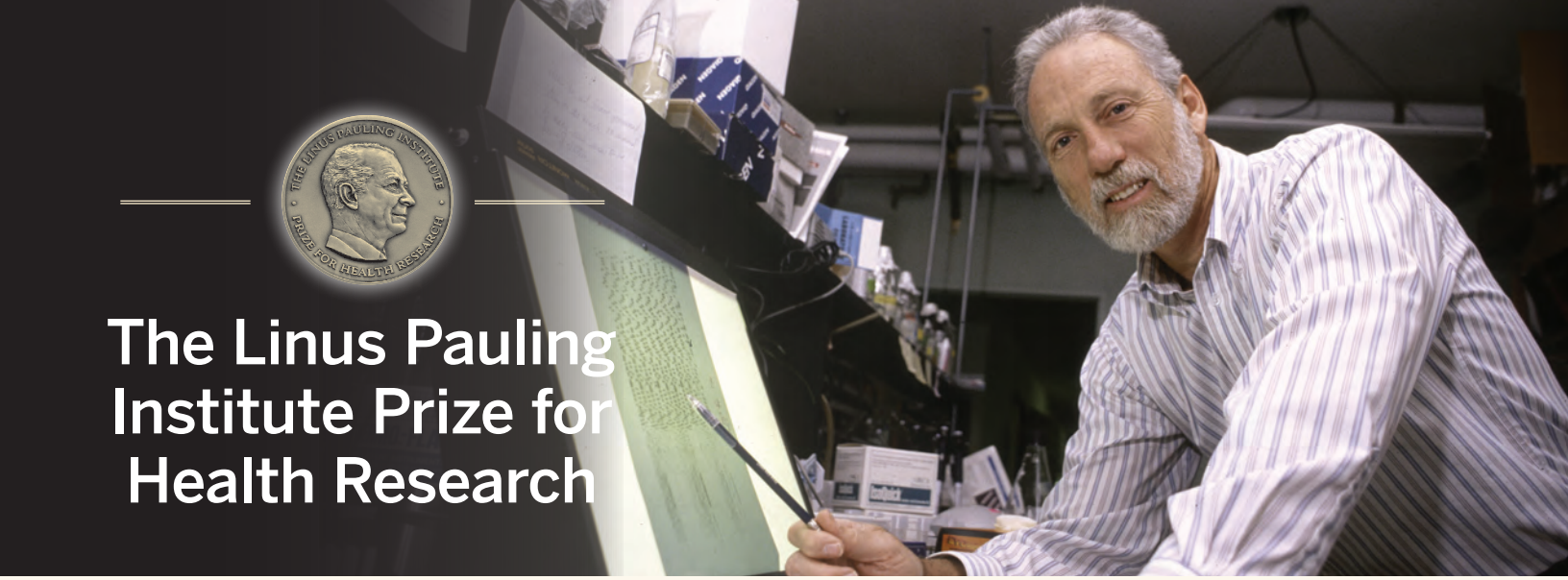
Eighty percent of Americans over 65 have at least one chronic disease, and many suffer from age-related declines in the quality of life.

• **John Groopman** (Johns Hopkins University) gave an overview of cancer and Bailey's work on aflatoxin and chlorophyll. Although about 50% of global cancer deaths occur in Asia and only 13% in North America, most research has focused on cancers relevant to North America and Europe. From 1991 to 2011, the incidence of cancer was lower than expected, primarily due to the declining use of tobacco. Liver cancer, which mainly afflicts those under 50 years old, remains the second leading cause of global cancer deaths. The dietary substitution of rice for corn (which is more likely to contain aflatoxin) beginning in about 1985 correlates with the dramatic reduction in liver cancer, and Bailey played a central role in developing and understanding dietary phytochemical interventions.

The Public Symposium on Saturday morning featured two of LPI's Principal Investigators: **Balz Frei**, who discussed LPI's "Rx for Health" (http://lpi.oregonstate.edu/rx-health) and **Tory Hagen**, who talked about increasing healthspan.

• As **Balz Frei** (LPI) noted, micronutrients themselves provide neither energy nor calories but are important in energy conversion, cell and tissue functions, the immune system, metabolism, and growth and development. There is a difference between frank deficiencies that can lead to disease and death, and inadequacy in which biological functions are suboptimal. Deficiencies of iron, zinc, vitamin A, and iodine are widespread globally, and even a large percentage of Americans do not meet the RDAs for several micronutrients. Generally, our intake of B vitamins is adequate because of food enrichment and fortification, but the intakes of "shortfall nutrients" like calcium, magnesium, potassium, and vitamins A, C, D, and E are low. Even a "perfect" diet does not provide adequate amount of vitamins D and E, potassium, and choline. Most people do not meet the recommended Dietary Guidelines for Americans for consumption of whole grains, fruit, vegetables, seafood, and fiber, and exceed the recommended intake of saturated fats, added sugars, refined grains, and sodium. Weight loss, the DASH diet, and daily aerobic exercise all reduce blood pressure more than the reduction of dietary sodium. The Physicians' Health Study II, a long-term, large-scale, randomized trial to assess interventions on disease risk, found that a daily multivitamin/mineral significantly reduced the incidence of total cancers among male physicians, a presumably well-nourished group. It is confidently estimated that a large percentage of chronic diseases are preventable by diet and lifestyle.

• Life expectancy was almost constant until 1900. By WW II, it had doubled in the US and is 82 years today. As **Tory Hagen** (LPI) noted, the increase is due largely to better sanitation, antibiotics, vaccinations, perinatal care, and drugs. Once-lethal diseases have now become chronic diseases that limit healthspan—that portion of your life when you are free from debilitating disease. Eighty percent of Americans over 65 have at least one chronic disease, and many suffer from age-related declines in the quality of life. Neurodegenerative diseases are common in people over 80, and older adults often experience weight loss, exhaustion, weakness, and slowness due to declines in cardiac function, nerve conduction, hearing, breathing capacity, and renal blood flow. Only about 15-25% of the longevity quotient is genetically determined; the majority is influenced by the environment, including diet. Five modifiable risk factors decrease longevity: 1) sedentary lifestyle, leading to frailty; 2) hypertension, leading to kidney and heart failure; 3) obesity, leading to cancer and cognitive disorders; 4) diabetes, leading to cardiovascular and cognitive declines; and 5) smoking. Many factors are associated with increased longevity, including marriage, frequent social contacts, moderate alcohol intake, and moderate physical activity, which is beneficial for all age groups. Restricting calories improves health and longevity, but antioxidants don't significantly extend lifespan or lower oxidative damage in old animals. Hundreds of candidates to extend healthspan are being examined, including dark chocolate, coffee, red wine constituents, berries, sulfur-containing compounds in cruciferous vegetables, and caloric restriction mimetics like rapamycin. Even low levels of stress may be beneficial by inducing a positive biological response—a concept known as "hormesis." LPI



The Linus Pauling Institute Prize for Health Research

The eighth Linus Pauling Institute Prize for Health Research was presented posthumously to Dr. George Bailey, Oregon State University distinguished professor emeritus and former LPI principal investigator. Dr. Bailey's widow, Carol, accepted the medal from LPI Director Balz Frei at the Diet and Optimum Health Conference banquet on Friday, September 11.

The Prize recognizes innovation and excellence in research on the roles of micronutrients (vitamins and minerals) and phytochemicals in promoting optimum health and preventing or treating disease and successful efforts to disseminate knowledge to enhance public health. As can be seen from the summaries of presentations at the Diet and Optimum Health Conference on pages 5, 7, and 8, Dr. Bailey developed the trout model to enable large-scale and economical studies with exceptional statistical power to detect the effects of low-dose exposure to toxins and carcinogens.

Dr. Bailey earned his Ph.D. in biochemistry from the University of California-Berkeley and joined Oregon State University in 1979, initially serving as an assistant professor of Food Science and Technology. He was the director of OSU's Marine and Freshwater Biomedical Sciences Center, sponsored by the National Institute of Environmental Health Sciences, from 1985 to 2002. He then became a Principal Investigator in LPI and continued to work on dose-response issues in carcinogenesis, especially focused on aflatoxin and polycyclic aromatic hydrocarbons, which are ubiquitous environmental carcinogens.

Working with trout, Dr. Bailey and his colleagues were able to assess the effect of very low doses of carcinogens, a feat not practical with rodents. Trout are relatively easy and economical to maintain, and huge numbers—tens of thousands—can be used in experiments for high statistical power. Their metabolism of and response to carcinogens is similar to people, so results may be reliably extrapolated. Because of the trout's sensitivity to carcinogens, low incidence of spontaneous tumors, and very high statistical power attained with large numbers of fish, Dr. Bailey was able to more precisely estimate human cancer risk from some carcinogens at the doses we are exposed to environmentally.

Aflatoxin is a carcinogenic substance formed by mold that grows on improperly stored grains. It's responsible for a high incidence of liver cancer in regions of China where people are unavoidably exposed dietarily. Aflatoxin also causes liver cancer in trout. Dr. Bailey and his colleagues showed that co-administering chlorophyll or its derivative chlorophyllin with aflatoxin protected trout from liver cancer. Chlorophyll binds to aflatoxin in the gut, preventing its absorption into the blood stream and subsequent delivery to the liver. The chlorophyll-aflatoxin complex is then harmlessly excreted. These results led to a clinical trial conducted by Drs. Bailey and Thomas Kensler in China in which people exposed dietarily to aflatoxin took a chlorophyllin supplement or placebo three times a day before meals for four months. Biomarkers of DNA damage—presumably indicative of cancer risk—were measured in urine after three months and found to be decreased by about half in those who got the chlorophyllin, suggesting that their risk for liver cancer was substantially decreased.

As Balz Frei noted, "George pioneered the use of rainbow trout as a model for human cancer, which lead to a new paradigm of assessing cancer risk at doses relevant to human exposure. And we are also honoring George for his pioneering work using dietary approaches and phytochemicals such as chlorophyll(in) and indole-3-carbinol for cancer chemoprevention, which has the potential to dramatically reduce rates of hepatocellular carcinoma, the third leading cause of cancer mortality worldwide, as well as colon and other cancers in the US and around the world."

LPI principal investigator Dave Williams, who worked with Dr. Bailey for many years, said, "For some reason what really sticks with me is the memory of his hands. I would look at those long, almost bony, fingers and marvel at the different tasks they could perform. Taking apart an old beat-up 1940's pickup truck engine and transmission, then restoring it to pristine condition; picking the banjo at a level good enough to play with the Sawtooth Mountain Boys; deftly casting a fly with a rod so that it would drop right in front of a large trout; hitting a 140-yard 8 iron to within a few feet from the pin; gripping the poles as he glided down the ski slope; and, of course, writing many impactful scientific publications and successful NIH grants." LPI

Selected Recent Publications by LPI Scientists

Summarized by Stephen Lawson, LPI Administrative Officer

LPI scientists in **boldface**

Abstracts of most of these papers can be found in PubMed (http://www.ncbi.nlm.nih.gov/pubmed)

Healthy Aging Program

Kerr DC, Zava DT, Piper WT, Saturn SR, **Frei B**, and **Gombart AF**. (2015) Associations between vitamin D levels and depressive symptoms in healthy young adult women. *Psychiatry Res.* **227**:46-51

Vitamin D_3 levels in serum were measured twice in 185 healthy, young college undergraduates in the Pacific Northwest during fall, winter, and spring. Almost half of the subjects were insufficient in vitamin D (<30 ng/ml), and lower vitamin D levels significantly predicted clinical depression.

Magnusson KR, Hauck L, Jeffrey BM, **Elias V**, Humphrey A, Nath R, Perrone A, and Bermudez LE. (2015) Relationships between diet-related changes in the gut microbiome and cognitive flexibility. *Neuroscience*. **300**:128-140

The authors fed mice either high-fat, high-sucrose, or normal chow diets for five weeks and then examined their cognitive and memory performance. Fecal samples were collected and assayed for bacteria to find out if the diets significantly affected the various bacterial populations in the gut. Long- and short-term memory were impaired in the mice fed the high-sucrose diet, and the microbiome (the collection of various populations of bacteria) was significantly altered by both the high-fat and, especially, the high-sucrose diets, suggesting that high intakes of sugar or fat may contribute to impaired cognitive function.

Pride H, **Yu Z**, **Sunchu B**, **Mochnick J**, Coles A, Zhang Y, Buffenstein R, Hornsby PJ, Austad SN, and **Pérez VI**. (2015) Long-lived species have improved proteostasis compared to phylogenetically-related shorter-lived species. *Biochem. Biophys. Res. Commun.* **457**:669-675

Autophagy is the process by which cells get rid of large molecular aggregations and damaged organelles. It complements the cellular ubiquitin/proteasome process that removes damaged and misfolded proteins. The authors examined these processes in cells from related species with very long (naked mole rats, sugar gliders, and little brown bats) or short (mice, opossums, and evening bats) lifespans. Autophagy was enhanced in the longer-lived species, as was proteasome activity, except for bats. The authors concluded that maintaining proteins in their functional state correlates with longevity.

Keith D, **Finlay L**, **Butler J**, **Gomez L**, **Smith E**, **Moreau R**, and **Hagen T**. (2014) Lipoic acid entrains the hepatic circadian clock and lipid metabolic proteins that have been desynchronized with advanced age. *Biochem. Biophys. Res. Commun.* **450**:324-329

Lipid metabolism changes with advancing age; these changes are associated with metabolic syndrome and other dyslipidemias. Our circadian clocks that synchronize behavior and biology with the day/night cycle also become less precise with advanced age. The authors found that lipoic acid fed for two weeks to old rats remediated dyslipidemias, partly through the normalization of the circadian clock.

Roberts BR, Lim NKH, McAllum EJ, Donnelly PS, Hare DJ, Doble PA, Turner BJ, Price KA, Lim SC, Paterson BM, Hickey JL, **Rhoads TW**, **Williams JR**, Kanninen KM, Hung LW, Liddell JR, Grubman A, Monty J-F, Llanos RM, Kramer DR, Mercer JFB, Bush AI, Masters CI, Duce JA, Li Q-X, **Beckman JS**, Barnham KJ, White AR, and Crouch PJ. (2014) Oral treatment with CuII(atsm) increases mutant SOD1 *in vivo* but protects motor neurons and improves the phenotype of a transgenic mouse model of amyotrophic lateral sclerosis. *J. Neuroscience* **34**:8021-8031

A mouse that mimics amyotrophic lateral sclerosis (ALS) has been used to study the disease and the mutations to an antioxidant enzyme, copper/zinc superoxide dismutase, that are associated with a fraction of human cases. The mutated enzyme loses its affinity for zinc, and the resultant zinc-deficient enzyme generates reactive molecules that are toxic to motor neurons. Superoxide dismutase that is copper deficient has also been found in mice that mimic ALS. In this new experiment, when a copper-containing compound, CuII(atsm), was fed to the mice, the amount of mutant superoxide dismutase increased and, surprisingly, resulted in improved physical function and survival. The authors found that the extra copper normalized the copper content in the copper-deficient superoxide dismutase in motor neurons in the spinal cord, thus stabilizing the enzyme and promoting its normal function.

Zamzow DR, **Elias V**, **Legette LL**, **Choi J**, **Stevens JF**, and **Magnusson KR**. (2014) Xanthohumol improved cognitive flexibility in young mice. *Behav. Brain Res.* **275**:1-10

An age-related increase in palmitoylation, a chemical modification of proteins, results in cognitive deficits. The authors examined the effect of xanthohumol, a phytochemical from hops used in beer manufacture, on protein palmitoylation in young and old mice. Although xanthohumol did not affect palmitoylation status, it did improve cognitive flexibility of young, but not old, mice on a phytoestrogen-deficient diet.

Fok WC, Chen Y, Bokov A, Zhang Y, Salmon AB, Diaz V, Javors M, Wood WH 3rd, Zhang Y, Becker KG, **Pérez VI**, and Richardson A. (2014) Mice fed rapamycin have an increase in lifespan associated with major changes in the liver transcriptome. *PLoS One* **9**:e83988

Rapamycin is a naturally occurring antibiotic found in the soil of Easter Island. It's often used after transplant surgery to help prevent organ rejection and functions as a caloric restriction mimetic—it produces effects in animals similar to those induced by caloric restriction. In this study, the investigators fed rapamycin to four-month-old mice and then analyzed gene expression in the liver when the mice were very old (25 months). Rapamycin increased the lifespan of both male and female mice and affected gene expression in females but only in half of the males. Affected genes include those involved in mitochondrial function (the source of cellular energy), protein ubiquitination (the process by which damaged proteins are targeted for degradation), and, surprisingly and unlike other interventions that extend lifespan, a reduction in resistance to oxidative stress.

Guo C and **Gombart AF**. (2014) The antibiotic effects of vitamin D. *Endocr. Metab. Immune Disord. Drug Targets*.**14**:255-266

Heliotherapy—exposing patients to sunlight—has been employed for centuries, although an understanding of the underlying molecular mechanism is just emerging. About ten years ago, it was found that vitamin D is effective in killing bacteria by stimulating the synthesis of an antimicrobial peptide called cathelicidin. Subsequently, this mechanism was found to explain the role of vitamin D in killing *M. tuberculosis*. Epidemiological studies have reported that vitamin D deficiency is associated with an increased risk for infections, including tuberculosis, respiratory tract infections, and influenza. Cathelicidins are synthesized in a variety of cells, including immune cells, where cathelicidins stimulate their activity. Low serum vitamin D levels hasten the progression of disease in HIV-infected subjects. A randomized, double-blind, placebo-controlled study found that 1,200 IU/day of supplemental vitamin D given to school children decreased the incidence of the flu. In another study, high levels of cathelecidin in kidney dialysis patients predicted a decrease in early mortality. However, several studies have found that vitamin D supplementation did not affect cathelicidin levels, suggesting that prior immune activation may be necessary. In healthy humans, a positive association between vitamin D and cathelicidin was observed only at levels of vitamin D less than 32 ng/ml in the blood. Also, the regulation of cathelicidin occurs only in humans and primates, making animal studies difficult to conduct.

Guo C, **Sinnott B**, Niu B, **Lowry MB**, **Fantacone ML**, and **Gombart AF**. (2014) Synergistic induction of human cathelicidin antimicrobial peptide gene expression by vitamin D and stilbenoids. *Mol. Nutr. Food Res.* **58**:528-536

The cathelicidin antimicrobial peptide is a small molecule made in the body that attacks pathogens like bacteria. Its production can be induced by vitamin D, niacin, curcumin from turmeric, and other substances. Using cell cultures, the authors screened a library of 446 molecules to find novel candidates that stimulate the synthesis of cathelicidin. When combined with vitamin D, resveratrol—found in grapes, peanuts, and wine—and pterostilbene—found in grapes and blueberries—synergistically induced the synthesis of cathelicidin.

Cardiometabolic Disease Prevention

Angelo G, **Drake VJ**, and **Frei B**. (2015) Efficacy of multivitamin/mineral supplementation to reduce chronic disease risk: A critical review of the evidence from observational studies and randomized controlled trials. *Crit. Rev. Food Sci. Nutr.* **55**:1968-1991

The authors reviewed the scientific literature for randomized controlled trials and observational studies on the effect of multivitamin/mineral (MVM) supplementation on the risk for chronic diseases like cardiovascular disease and cancer and age-related eye diseases like cataract and macular degeneration. Most studies have reported no significant effect of MVM supplements on chronic disease risk, with a few exceptions. The Physicians' Health Study II (PHS II), a study of over 14,000 male physicians followed for over 11 years, found that the use of a daily MVM decreased the risk for total cancers by 8%. Another large French study found that the intake of a daily supplement containing antioxidant vitamins and minerals for about 7.5 years was associated with a 30% reduction in the risk for cancer and all-cause mortality in men but not in women. While the PHS II found that a daily MVM was associated with a 9% reduction in the risk for total cataract, there was a 38% increase in the risk for age-related macular degeneration in men over 70.

Jump DB, **Depner CM**, **Tripathy S**, and **Lytle KA**. (2015) Impact of dietary fat on the development of non-alcoholic fatty liver disease in Ldlr-/- mice. *Proc. Nutr. Soc.* **18**:1-9

Fatty liver disease (steatosis) is quite common and associated with abdominal obesity. Simple non-alcoholic fatty liver disease (NAFLD) can progress to non-alcoholic steatohepatitis (NASH), which causes liver injury, chronic inflammation, oxidative stress, and fibrosis. The authors developed a mouse model that mimics human NASH. When these mice were fed a Western-type diet high in saturated and *trans* fat, sugar, and cholesterol, they developed NASH.

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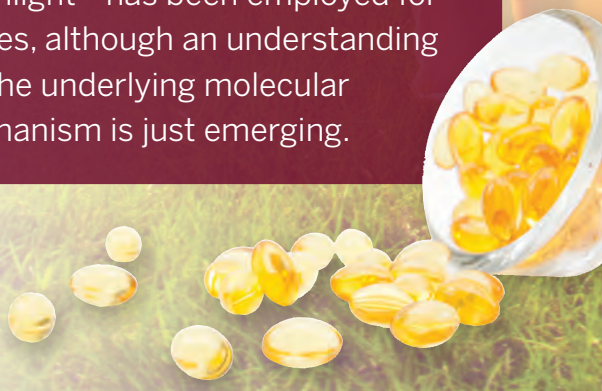
Heliotherapy—exposing patients to sunlight—has been employed for centuries, although an understanding of the underlying molecular mechanism is just emerging.



Continued from page 11 — Selected Publications

Supplementing their diet with the omega-3 fatty acids docosahexaenoic acid (DHA) and eicosapentaenoic aicd (EPA), found in fish oil, attenuated inflammation, oxidative stress, fibrosis, and dyslipidemia. DHA was especially effective.

Elie MR, **Choi J**, Nkrumah-Elie YM, Gonnerman GD, **Stevens JF**, and Tanguay RL. (2015) Metabolomic analysis to define and compare the effects of PAHs and oxygenated PAHs in developing zebrafish. *Environ. Res.* **140**:502-510

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants and carcinogens formed from the burning of carbon-containing fuels. We are exposed to them in the air and in food. The authors exposed young zebrafish, a good experimental model for vertebrate development, to two PAHS and looked for developmental defects. After five days of exposure, the zebrafish had impaired protein synthesis, mitochondrial function, neural and vascular development, and cardiac function.

Legette L, Karnpracha C, **Reed RL**, **Choi J**, **Bobe G**, Christensen JM, Rodriguez-Proteau R, Purnell JQ, and **Stevens JF**. (2014) Human pharmacokinetics of xanthohumol, an antihyperglycemic flavonoid from hops. *Mol. Nutr. Food Res.* **58**:248-255

Xanthohumol, a phytochemical from hops used in the manufacture of beer, has been shown in rodents to possess anti-inflammatory, antihyperglycemic, and antioxidant functions. Its ability to lower blood glucose and attenuate lipid dysfunction suggests potential therapeutic benefit in type 2 diabetes and metabolic syndrome. The authors gave three doses (20, 60, or 180 mg) to 24 healthy men and 24 healthy women and then determined maximum blood levels of xanthohumol (45, 67, and 133 micrograms/L, respectively). The concentration of xanthohumol in the blood peaked about one hour after ingestion and again after another three to four hours. About half of the xanthohumol was gone from the blood in 18-20 hours. No adverse effects of xanthohumol were observed, and the results of this study show that xanthohumol metabolism is similar in rodents and humans.

Lebold KM and **Traber MG**. (2014) Interactions between alpha-tocopherol, polyunsaturated fatty acids, and lipoxygenases during embryogenesis. *Free Radic. Biol. Med.* **66**:13-19

In this review article, the authors address the importance of vitamin E, primarily alpha-tocopeherol, in early development. Vitamin E protects polyunsaturated fatty acids like arachidonic acid and docosahexaenoic acid—critical for brain development—from oxidation. Vitamin E deficiency depletes vitamin C, and vitamin C supplementation can regenerate vitamin E from its oxidized form. Alpha-tocopherol deficiency in zebrafish embryos resulted in impaired neurological development, anatomical malformations, and increased mortality. Since the USDA estimated that 96% of American women do not meet the dietary recommended intake for vitamin E, this could have implications for the development of their embryos should they become pregnant while getting insufficient vitamin E. Additionally, a Chinese study found that infants with higher vitamin E status had better cognitive function at two years of age.

Murer SB, Aeberli I, Braegger CP, Gittermann M, Hersberger M, **Leonard SW**, **Taylor AW**, **Traber MG**, and Zimmermann MB. (2014) Antioxidant supplements reduced oxidative stress and stabilized liver function tests but did not reduce inflammation in a randomized controlled trial in obese children and adolescents. *J. Nutr.* **144**:193-201

Forty-four obese children and adolescents (average age of 13 years) were given daily antioxidants (400 IU of vitamin E, 500 mg of vitamin C, and 50 micrograms of selenium) or placebo for four months. Various metabolic parameters, including oxidative stress, inflammation, glucose levels, lipid profiles, and liver enzymes, were measured in blood samples at baseline and after four months of supplementation. Antioxidant supplementation increased the level of antioxidants in blood, decreased oxidative stress, and improved liver function but did not affect inflammation.

Traber MG. (2014) Vitamin E inadequacy in humans: Causes and consequences. *Adv. Nutr.* **5**:503-513

Vitamin E is a family of eight forms, but the human requirements are based on alpha-tocopherol, which is the only form that can reverse deficiency symptoms. Over 90% of American men and women do not consume 12 mg of vitamin E daily, the EAR (estimated average requirement). It's difficult to assess the impact of vitamin E inadequacy—circulating levels in blood do not correlate well with dietary intakes because of age-related changes in lipoproteins that carry vitamin E. Certain genetic defects and fat malabsorption problems may result in severe vitamin E deficiency, characterized by cardiomyopathy and neurological abnormalities, including ataxia. Symptoms of mild deficiency may be subtle, although studies in zebrafish suggest that vitamin E is crucial for normal brain and neuronal development. Therefore, vitamin E adequacy is imperative for pregnant women. Vitamin E protects fatty acids in the brain from oxidation and also protects cognitive function. Some studies found that patients with Alzheimer's disease had lower vitamin E plasma levels and that vitamin E supplementation slowed the progression of the disease.

Michels AJ and **Frei B**. (2013) Myths, artifacts, and fatal flaws: Identifying limitations and opportunities in vitamin C research. *Nutrients* **5**:5161-5192

Vitamin C has been a focus of research since its discovery as the anti-scorbutic factor in 1932, yet, surprisingly, much about its role in health and disease is still unknown. Vitamin C is difficult to manage in cell culture studies because media components like metals cause its oxidation. Most human cells in culture are scorbutic because vitamin C is not routinely supplied to them, and permanent cell culture lines have been grown without vitamin C for many generations, possibly affecting their metabolism and responses to experimental interventions. Most animals synthesize vitamin C, so experimental animal models must be chosen

with care. In humans, age and genetic polymorphisms can affect how much vitamin C is absorbed into the blood stream from the gut. Pharmacokinetic studies of vitamin C have illuminated some issues of absorption and excretion of vitamin C taken orally or intravenously, but these were performed in a small number of young, healthy subjects and may not usefully indicate the relationship between intake and resultant levels in all organs and tissues, especially in older or ill subjects. There may be a poor correlation between assessment of dietary intake of vitamin C, which is problematic, and plasma levels, further confounding the interpretation of human studies.

Cancer Prevention and Intervention

Perera T, Young MR, Zhang Z, Murphy G, Colburn NH, Lanza E, Hartman TJ, Cross AJ, and **Bobe G.** (2015) Identification and monitoring of metabolite markers of dry bean consumption in parallel human and mouse studies. *Mol. Nutr. Food. Res.* **59**:795-806

The Polyp Prevention Trial (PPT) was a four-year randomized trial with over 1,900 men and women who had prior colorectal adenomas removed and either consumed their usual diet or ate a low-fat, high-fruit and -vegetable, and high-fiber diet. Participants who reported increased consumption of dry beans (e.g., baked, kidney, pinto lima, black, and navy beans) had a reduced risk for advanced adenoma recurrence. The Legume Inflammation Feeding Experiment (LIFE) study examined the effect of a diet high in dry beans on inflammation and insulin sensitivity in healthy men. The authors identified several bean metabolites in the serum of participants in the PPT and LIFE bean consumption studies and in mice fed a diet enriched with an extract of navy beans that may serve as biomarkers of bean consumption. These biomarkers will be useful in quantitative and precise studies on the prevention of colorectal cancer by beans.

Atwell LL, **Hsu A**, **Wong CP**, **Stevens JF**, Bella D, **Yu TW**, Pereira CB, Lohr CV, Christensen JM, Dashwood RH, **Williams DE**, Shannon J, and **Ho E.** (2015) Absorption and chemopreventive targets of sulforaphane in humans following consumption of broccoli sprouts or a myrosinase-treated broccoli sprout extract. *Mol. Nutr. Food Res.* **59**:424-433

Sulforaphane is a phytochemical found in cruciferous vegetables like broccoli, cabbage, and Brussels sprouts that has anticancer effects in cell cultures and rodents. In this study, the authors determined the absorption of sulforaphane in people. Cruciferous vegetables contain glucoraphanin; when they are chopped or chewed, an enzyme called myrosinase is released that converts glucoraphanin to sulforaphane, which is then absorbed into the blood stream. Twenty healthy adults aged 19 to 50 were given a myrosinase-treated broccoli sprout extract or fresh broccoli sprouts. The levels of sulforaphane metabolites in the urine and plasma of the subjects who consumed fresh broccoli sprouts were three to five times higher than those in subjects who got the broccoli sprout extract, although putative anticancer mechanisms in both groups were not significantly affected by the doses used.

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Sulforaphane is a phytochemical found in cruciferous vegetables like broccoli, cabbage, and Brussels sprouts that has anticancer effects in cell cultures and rodents.

Continued from page 13 — Selected Publications

Wong CP, Rinaldi NA, and **Ho E.** (2015) Zinc deficiency enhanced inflammatory response by increasing immune cell activation and inducing IL6 promoter demethylation. *Mol. Nutr. Food Res*. **59**:991-999

Chronic inflammation, an abnormally persistent immune response, is associated with many age-related diseases, including arthritis, atherosclerosis, diabetes, and cancer. In this study, biomarkers of inflammation were increased in zinc-deficient cells in culture and in old mice with age-related declines in zinc status, indicating abnormal immune activation. Methylation, a normal chemical modification of genes and proteins, was also decreased in zinc-deficient cells and mice.

Rajendran P, Dashwood WM, Li L, **Kang Y**, Kim E, Johnson G, Fischer KA, Lohr CV, **Williams DE**, **Ho E**, Yamamoto M, Lieberman DA, and Dashwood RH. (2015) Nrf2 status affects tumor growth, HDAC3 gene promoter associations, and the response to sulforaphane in the colon. *Clin. Epigenetics* **7**:102. doi:10.1186/s13148-015-0132-y

Nuclear factor erythroid 2 (NF-E2)-related factor 2 (Nrf2) is a transcription factor that migrates into the cell nucleus when oxidative stress occurs. Once in the nucleus, Nrf2 stimulates the expression of antioxidant genes. The authors treated normal mice and mice deficient in Nrf2 with a colon carcinogen, followed by dietary exposure to sulforaphane from cruciferous vegetables. As expected, sulforaphane inhibited colon tumorigenesis and decreased tumor size in normal mice, accompanied by inhibition of histone deacetylase (HDAC) activity and increased synthesis of a tumor suppressor protein called p16. Surprisingly, the Nrf2-deficient mice had fewer tumors, less HDAC activity, and lower p16 levels than normal mice through mechanisms yet to be determined. In people given sulforaphane as a broccoli sprout extract, HDAC activity decreased and p16 levels increased in peripheral blood mononuclear cells.

Watson GW, Wickramasekara S, Fang Y, Palomera-Sanchez Z, Maier CS, **Williams DE**, Dashwood RH, **Pérez VI**, and **Ho E.** (2015) Analysis of autophagic flux in response to sulforaphane in metastatic prostate cancer cells. *Mol. Nutr. Food Res*. **59**:1954-1961

Autophagy is a process in which cellular components like damaged organelles or proteins are delivered to the lysosome for degradation. Autophagy is dysregulated in cancer cells; stimulating autophagy may have anticancer benefits. The authors treated metastatic prostate cancer cells with sulforaphane to assess its effect on autophagy. At physiologically relevant concentrations, sulforaphane did not affect autophagy in either acute or prolonged exposures. At much higher concentrations, sulforaphane did induce autophagy and subsequent cell death.

Atwell LL, **Beaver LM**, Shannon J, **Williams DE**, Dashwood RH, and **Ho E.** (2015) Epigenetic regulation by sulforaphane: Opportunities for breast and prostate cancer chemoprevention. *Curr. Pharmacol. Rep*. **1**:102-111

Sulforaphane, an anticancer phytochemical in cruciferous vegetables, exerts its effects through several mechanisms, including genetic and epigenetic processes. Epigenetics refers to changes in gene expression (protein synthesis) not caused by changes to DNA itself. Sulforaphane works against prostate cancer cells through epigenetic mechanisms, including activation of tumor suppressor genes (probably by the inhibition of histone deacetylases [HDACs]), inhibition of tumor-promoting genes, induction of apoptosis (programmed cell death), induction of cellular antioxidant defenses, and a reduction of inflammation. In breast cancer cells, sulforaphane also inhibited HDACs, enhanced anti-oxidant defenses, and induced apoptosis. More research is needed to understand fully the effect of cooking on sulforaphane stability, the distribution of sulforaphane and its metabolites to various tissues, the effect of individual variability on sulforaphane metabolism, and the role of gut bacteria in sulforaphane metabolism.

Watson GW, **Beaver LM**, **Willams DE**, Dashwood RH, and **Ho E.** (2013) Phytochemicals from cruciferous vegetables, epigenetics, and prostate cancer prevention. *AAPS J*. **15**:951-961

Prostate cancer is very common in the United States, accounting for about 30% of cancer diagnoses. Epidemiological studies suggest that diet is an important determinant of prostate cancer risk; men who consume lots of cruciferous vegetables have less risk. Mechanistic research has focused on two anticancer phytochemicals in cruciferous vegetables: sulforaphane and indole-3-carbinol (I3C), both of which are produced from precursors by the enzyme myrosinase, which is released when the vegetables are chopped or chewed. Sulforaphane induces phase 2 enzymes, which detoxify and chemically prepare toxins and carcinogens for elimination; suppresses aberrant cell-signaling pathways in cancer cells; induces apoptosis (programmed cell death), and inhibits histone deacetylases (HDACs), which are overactive in cancer cells. I3C also stimulates detoxification enzymes, induces apoptosis, inhibits androgen signaling, and indirectly inhibits HDAC activity. LPI

DEVELOPMENTS

A Lasting Legacy of Nutritional Science

In my previous column for the LPI research newsletter, I asked readers to share their stories about Linus Pauling. I heard over and over how approachable and charming he was, as well as what a great sense of humor he had. His impact on the lives of many people around the world is immeasurable, and we're fortunate that he was willing to persevere in his vitamin C research despite skepticism from the medical community.

What science has shown us in the years since is what many of you know first-hand—Dr. Pauling's revolutionary views on the importance of orthomolecular medicine are far from discredited.

Almost 20 years ago, the Linus Pauling Institute of Science and Medicine moved to Oregon State University, where it became the Linus Pauling Institute. Institute Director Balz Frei was hired in 1997, and the first three principal investigators arrived at the Institute in 1998, following a national search for innovative scientists with expertise in micronutrients and health.

By 2011, when the Institute moved into the Linus Pauling Science Center, a state-of-the-art research facility that brought together all of the Institute's principal investigators, three core areas of research had evolved: Healthy Aging (Healthspan), Cancer Prevention and Intervention, and Cardiometabolic Disease Prevention. Outreach programs like the Micronutrient Information Center, the Healthy Youth Program, and this newsletter allow the Institute to share the results of that research with people in the community and around the world.

As we look ahead to the Institute's 20th anniversary at OSU next year, the Institute has 12 principal investigators, four adjunct faculty, and 27 research staff and graduate students working on research projects in orthomolecular medicine. From vitamin and supplement studies to investigating the impact of phytochemicals and the role of the microbiome, our researchers are demonstrating just how critical the right molecule at the right concentration at the right time can be for our health. These molecular studies are enabled by technological advances in mass spectrometry and other instruments and would not have been possible years ago.

None of these advances at the Linus Pauling Institute would have been possible without the support of people like you. As we get ready to celebrate the Institute's 20th anniversary at OSU, I hope you will consider making a gift in honor of Dr. Pauling's vision and to support future advances in orthomolecular medicine.

Keep up with news from the Institute between newsletters on the Linus Pauling Institute's blog (http://blogs.oregonstate.edu/LinusPaulingInstitute) and on Facebook at www.Facebook/LinusPaulingInstitute. LPI

Marlys Amundson
Director of Development

Photo by Jim Carroll Photography

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Look for these informative articles inside!

GIVING to the Linus Pauling Institute

Gifts in support of research efforts can be made at any time. Checks should be payable to *OSU Foundation for Linus Pauling Institute.* Information on giving is available through the OSU Foundation, **1-800-354-7281**, or by writing to the Institute.

Create a lasting legacy at the Linus Pauling Institute

A "life-income gift" guarantees an income stream for life. Additional benefits may include:

- Increase your income
  (a portion may even be tax-free)
- Receive an immediate charitable deduction
- Reduce capital gains taxes
  (if the gift is funded with appreciated property or securities)
- Reduce estate tax

Life-income gifts are simple and flexible. You transfer cash, stocks, real estate, or other property to fund the life-income gift. You (or whomever you designate) receives the income stream for life or for a set number of years. Payments may begin immediately or are deferred to a future date, such as retirement. Ultimately, your life-income gift will benefit the Linus Pauling Institute. Many life-income donors direct their gift to establish an endowed fund in their name, creating a lasting legacy.

To learn more about gifts that pay income, **please click http://bit.ly/1PAAbIM**, or call me at **800-354-7281** for a personalized illustration.

Sincerely,
Jeff Comfort, Vice President, Principal Gifts and Gift Planning

Special thanks to *Barbara McVicar for editorial assistance and photographs and to authors of signed articles.*