



OSU Oregon State University

# The Linus Pauling Institute

R E S E A R C H N E W S L E T T E R



## From the Director

*Balz Frei, Ph.D.  
LPI Director and Endowed Chair  
Professor of Biochemistry  
and Biophysics*

Much has happened at the Linus Pauling Institute since the last Research Newsletter. In May, we held our fifth Diet and Optimum Health Conference in Portland, Oregon, with a record attendance of over 250 scientists from all over the world. At the conference, we presented the LPI Prize for Health Research to Dr. Michael Holick, a professor of medicine, physiology, and biophysics at the Boston University School of Medicine who revolutionized the understanding of vitamin D and its role in disease prevention. A summary of Dr. Holick's presentation and all the other talks at the conference can be found in this Newsletter. In August, we held our 11<sup>th</sup> annual LPI retreat at the Oregon Garden Resort near Salem, Oregon, where all the faculty, students, and research staff in LPI presented their latest findings and discussed new research directions and projects.

On September 25<sup>th</sup>, we celebrated the construction launch of the Linus Pauling Science Center, the future home of LPI at Oregon State University. Over 300 people attended the festivities, which featured numerous speakers. I had the honor of delivering the opening remarks, in which I described my first meeting with Dr. Linus Pauling on October 17, 1989, at the Linus Pauling Institute of Science and Medicine in Palo Alto, California. That also was the day of the 6.9-Richter scale Loma Prieta earthquake, which made my first encounter with Dr. Pauling a truly "earth-shaking" experience. I likened this event to the construction of the Linus Pauling Science Center, which also is an historic event—and will cause a seismic shift—for LPI and OSU. I went on to explain that the state-of-the-art research facilities that LPSC will provide for the Institute will free us "to rigorously pursue our mission to determine the role of diet, micronutrients, and lifestyle in preventing chronic disease and promoting

*continued on page 2*



## LPI Diet and Optimum Health Conference

*Stephen Lawson  
LPI Administrative Officer*

The fifth biennial LPI Diet and Optimum Health Conference, co-sponsored by the Oxygen Club of California and Oregon Health & Science University, convened in Portland from May 13<sup>th</sup> to 16<sup>th</sup>. The conference featured 22 speakers from around the world and was organized into five sessions:

- *Healthy Aging: Neurocognitive Disorders and Molecular Mechanisms*
- *Micronutrients and Immune Function*
- *Vitamin K: New Functions and Mechanisms of Action*
- *Diet and Lifestyle in Metabolic Syndrome and Cardiovascular Diseases*
- *Epigenetics, Diet, and Disease*

The conference opened on Wednesday afternoon with a special session of oral presentations selected from the many abstracts submitted for posters. Additionally, finalists for the Young Investigator Awards, sponsored jointly by LPI and the Oxygen Club of California, gave short presentations. Heather Kuiper, Alexander Michels, and Mansi Parasramka were selected for Awards, which were presented by Rod Dashwood of LPI and John Maguire of OCC at the banquet on Friday evening.

## Healthy Aging: Neurocognitive Disorders and Molecular Mechanisms

*Chaired by Tory Hagen (LPI) and Kathy Magnusson (Oregon State University)*

- **Kevin Pearson** (University of Kentucky) discussed healthspan extension by caloric restriction and resveratrol, a polyphenol thought to mimic the effects of caloric restriction. In mice, resveratrol did not extend lifespan but did produce some health benefits, such as decreased inflammation, improved aortic function, inhibition of cataracts, increased

*continued on page 3*

optimum health. By extending not only lifespan but also 'healthspan,' the Linus Pauling Institute will improve individual and public health, reduce human suffering, and help control the spiraling cost of health care. Our efforts in preventive medicine are critical for the health and economic vitality of cities, towns, and rural areas across the U.S., as communities seek to cope with an ever-increasing aging population."



Dr. Gerd Bobe

Furthermore, I am very pleased to announce that a new Principal Investigator, **Dr. Gerd Bobe**, joined the LPI faculty in October. Dr. Bobe comes to us from the National Cancer Institute, where he was a post-doctoral Cancer Prevention Fellow for the past four years. His research focuses on identifying biomarkers in humans and animal models that are associated with colorectal cancer and are susceptible to modification by diet or dietary supplements, including micronutrients and flavonoids from fruits and vegetables. Dr. Bobe has a Master of Public Health with concentration in Public Health Nutrition from Johns Hopkins University and a Ph.D. in Animal Nutrition from Iowa State University. Dr. Bobe will be joining our Cancer Chemoprotection Program, which is directed by **Dr. Rod Dashwood**. We are excited about Dr. Bobe joining LPI and extend a warm welcome to his family.

Finally, LPI faculty have been exceptionally successful in securing several grants from the National Institutes of Health funded by the American Recovery and Reinvestment Act of 2009. **Dr. Joseph Beckman**, the Ava Helen Pauling Chair, was awarded a supplement to his Environmental Health Sciences Center grant, "Reducing susceptibility to environmental stress throughout the lifespan." The primary use of the funds will be to recruit new center investigators, support the career development of new faculty, and award pilot project grants to improve health and reduce environmental stress in older adults through diet and lifestyle. **Dr. Emily Ho**, a member of the Cancer Chemoprotection Program, was awarded a supplement to investigate "Dietary histone deacetylase inhibitors in prostate cancer prevention." The long-term goal of this project is to determine the mechanisms by which cruciferous vegetables decrease prostate cancer risk, including studies of the bioavailability in humans of dietary and supplemental sulforaphane, a dietary factor abundant in broccoli and other cruciferous vegetables. **Dr. Debbie Mustacich**, Research Assistant Professor, was awarded a grant to support her work on the "Prophylactic use of vitamin E for prevention of polycyclic aromatic hydrocarbon-induced damage." Polycyclic aromatic hydrocarbons (PAHs) are cancer-causing compounds produced as by-products of the burning of fossil fuels or biomass. The hypothesis underlying Dr. Mustacich's proposal is that vitamin E supplementation protects the liver and other organs

from DNA damage by increasing detoxification or elimination of PAHs and their metabolites from the body. **Dr. Maret Traber**, our "Goddess" of vitamin E, was awarded a supplement for her grant, "Mechanisms of vitamin E function studied in zebrafish." The goal of this project is to define molecular targets altered by vitamin E deficiency using zebrafish embryos, a unique experimental system that will allow Dr. Traber to answer why vitamin E is essential for reproduction and maintaining a healthy nervous system. And last but not least, **Dr. David Williams**, who was recently awarded the OSU Alumni Association Distinguished Professor Award, received a supplement for his grant "PAHs: new technologies and emerging health risks." The grant, a so-called Superfund Research Program, is a multi-project, \$12.3-million grant directed by Dr. Williams to investigate human health impacts from exposure to PAHs and to develop new technologies to assess exposure and risk. The supplement funded by the American Recovery and Reinvestment Act provides training and career development for **Dr. Sharon Krueger**, Research Assistant Professor, who will investigate "Environmental PAH mixtures as skin and transplacental carcinogens." Congratulations to all of these LPI investigators for their success in securing these competitive NIH grants! **LPI**

## Micronutrients and Violent Behavior

The "News Focus" section of the September 25<sup>th</sup>, 2009, issue of the journal *Science* featured an article on the use of micronutrient supplements to attenuate violence in prisons by inmates. The article cited Linus Pauling's seminal paper, "Orthomolecular Psychiatry," published in *Science* in 1968 that discussed "the treatment of mental disease by the provision of the optimum molecular environment for the mind."

Dr. Bernard Gesch recently initiated a randomized, double-blind clinical trial in a violent prison in Scotland to determine if daily supplementation with vitamins, minerals, and fatty acids will affect violent behavior. The trial is designed as a follow-up to an earlier study by Dr. Gesch in England that found prisoners who received supplemental micronutrients exhibited less violent behavior than non-supplemented prisoners. B vitamins, vitamin C, essential minerals and fatty acids, and other micronutrients affect brain function in many ways, including neuronal activity, synthesis of neurotransmitters, and, possibly, by reducing heavy metal burden. Many diets, including those in institutions, do not provide adequate amounts of important micronutrients, which may lead to anti-social behavior. If the putative value of micronutrient supplements can be confirmed, more institutions—not only prisons—worldwide may adopt this nutritional strategy to decrease violent behavior.





*Drs. Enrique Cadenas and Roland Stocker*

bone mineral density, and improved glucose tolerance. • **Tomas Prolla** (University of Wisconsin) noted that low-dose resveratrol mimics caloric restriction and retarded age-related decrements in mice. Mice fed resveratrol or calorically restricted had similar changes in gene expression, which were associated with protection against cardiac dysfunction and skeletal muscle aging. • **Craig Cooney** (University of Arkansas) has studied the epigenetic effects of diet on aging and diabetes in mice. “Epigenetics” refers to changes in gene expression without any changes to DNA. In a special mouse strain, coat color is affected epigenetically through dietary manipulation and is associated with glucose handling and insulin sensitivity. • **Carl Cotman** (University of California-Irvine) presented the results of a three-year study of cognition in beagles fed alpha-lipoic acid, carnitine, and vitamins C and E and/or environmentally enriched (exercise, play, social interaction, and cognitive experience). Beagles fed antioxidants and environmentally enriched scored best on cognitive tests. Antioxidants also inhibited beta-amyloid formation—associated with dementia—in the brain. • **David Kennedy** (Northumbria University) conducted two supplement studies showing that 1) men given a multivitamin/mineral supplement for five weeks reported increased vigor and less mental fatigue than unsupplemented men, and 2) working women taking a multivitamin/mineral supplement for nine weeks reported better mood and had increased cognitive performance and decreased homocysteine levels, a marker for heart disease. Additionally, in a small study, resveratrol increased cerebral blood flow and oxygen use in a dose-dependent manner. • Oxidative stress has been implicated in Alzheimer’s disease (AD). **D. Allan Butterfield** (University of Kentucky) uses proteomics to study proteins in the brain that are oxidatively modified in AD. The brain has high levels of oxygen and unsaturated fatty acids, low levels of antioxidants, and iron, making brain proteins vulnerable to oxidative modifications. Amyloid beta-peptide, associated with AD, also causes oxidative changes to brain proteins. Old beagles exhibit dementia and amyloid beta plaque formation, but supplementation with antioxidants and behavioral enrichment, including exercise, attenuated plaque formation and oxidative stress, leading to cognitive improvement.

## Micronutrients and Immune Function

*Chaired by Emily Ho (LPI)*

• Over one billion people worldwide are vitamin D deficient, leaving them vulnerable to bone problems, heart disease, autoimmune disorders, and infections. **Adrian Gombart** (LPI) discussed how vitamin D boosts the innate immune system, resulting in increased phagocytic activity and decreased inflammation by stimulating the production of an antimicrobial protein called cathelicidin. Low vitamin D levels are insufficient for the cathelicidin gene to be expressed. The development of a transgenic mouse model allows further investigation of cathelicidin activity. • The RDA for vitamin A is mainly based on its requirement for vision, but **Charles Stephensen** (University of California-Davis) argued that the role of vitamin A in immune function should be more carefully evaluated. Vitamin A deficiency, due to low intake, fat malabsorption problems, and frequent infections, is associated with high childhood mortality from infections. In clinical trials, supplementation decreased childhood mortality from infections by 30%, including measles and diarrheal diseases. However, very high vitamin A status in infancy may pre-dispose to asthma. • **Wafaie Fawzi** (Harvard) discussed the association between multiple micronutrient deficiencies and infectious diseases in Africa and Asia. In various studies, vitamin A decreased mortality from malaria, zinc decreased mortality from pneumonia and diarrheal diseases, high levels of maternal vitamin A decreased transmission of HIV to infants, multi-vitamins improved child growth and reduced the risk of mortality from or progression of HIV in women by 30%, and iron decreased the risk for anemia and improved growth and cognitive development in children, although children infected with malaria did worse with iron supplements.



*Dr. Tsuyako Saito*

## Vitamin K: New Functions and Mechanisms of Action

*Chaired by Maret Traber (LPI)*

• Vitamin K, a fat-soluble vitamin, is required for normal blood clotting and bone growth and is found in vegetable oils and green, leafy vegetables. **Sarah Booth** (Tufts) discussed the variability in vitamin K status among people, partly determined by estrogen status and genetic polymorphisms, but also due to variations in diet and absorption and antagonism with other fat-soluble vitamins. Since vitamin K poorly crosses the placenta and is low in breast milk, infants are at risk for vitamin K deficiency. • **Leon Schurgers** (University of Maastricht) addressed the cardioprotective function of vitamin K2. Vitamin K refers collectively to a group of related compounds: K1 is

*continued on page 4*



synthesized in plants and K2 is synthesized in bacteria and animals. A Dutch study found that high levels of K2—but not K1—were associated with protection against aortic calcification and a 50% lower risk for death from cardiovascular disease, probably due to K2's role in producing matrix Gla-protein, which may serve as a biomarker for cardiovascular disease. • **Kathleen Berkner** (Cleveland Clinic Foundation) noted that vitamin K is necessary for the carboxylation of the glutamate residue in protein that takes place in the liver. Carboxylation of proteins allows them to perform important physiological functions involved in blood clotting and calcium homeostasis. Studies of carboxylation have revealed that mutations of critical molecules cause disease, including pseudoxanthoma elasticum.

## Diet and Lifestyle in Metabolic Syndrome and Cardiovascular Diseases

*Chaired by Donald Jump (LPI) and Jeffrey Blumberg (Tufts)*

• **Christopher Newgard** (Duke) studies metabolites to understand mechanisms of human disease. In obese, insulin-resistant people, metabolism of branched-chain amino acids in proteins is altered, leading to increased insulin resistance. Western-type diets typically provide an



*OSU President Dr. Ed Ray addresses banquet attendees*

excess of these amino acids, leading to high levels of their detectable metabolites. In the context of a high-fat, high-calorie diet, too much protein may cause insulin resistance, associated with metabolic syndrome and diabetes.

• **Deborah Muoio** (Duke) pondered the association between weight gain, inactivity, and insulin resistance. Lipids accumulate in muscle tissue in obese people and, paradoxically, in athletes. In the sedentary obese, elevated lipids are incompletely oxidized, leading to insulin resistance. In athletes, these lipids provide the mitochondria in muscle cells with extra fuel. In mice fed a high-fat diet, exercise restored glucose tolerance (insulin sensitivity). • **Jonathan Purnell** (Oregon Health & Science University) compared various dietary regimens used to lose and control weight. Abdominal obesity is associated with glucose intolerance,

hypertension, diabetes, and heart disease. Low-calorie diets are successful in the short term but often fail long term. Metabolic studies have shown that low-fat, high-protein diets may be best for weight loss and that low-fat diets may be more effective in the obese. Weight loss achieved through dieting is usually modest, and the health benefits vary substantially among individuals. • A certain heritable phenotype of low-density lipoprotein (LDL-B) is a marker for both atherogenesis and a metabolic profile associated with altered triglyceride metabolism. **Ronald Krauss** (Children's Hospital Oakland Research Institute) explained how LDL-B is modified by various factors, including age, gender, and diet. A low-fat, high-carbohydrate diet induces LDL-B, and carbohydrate restriction in overweight people improves the lipid-related risk for heart disease. • **Ernst Schaefer** (Tufts) reviewed dietary intervention studies on heart disease risk. Replacing animal fat with vegetable oil, increasing the intake of fish or fish oil, decreasing caloric intake (especially from sugar and corn syrup), and increasing exercise all help lower the risk for heart disease. Docosahexaenoic acid (DHA), an omega-3 fatty acid found in fish, may reduce the risk for arrhythmia and Alzheimer's disease. High-fructose corn syrup increases lipogenesis, triglycerides, and the risk for heart disease. • The "antioxidant conundrum"—why have clinical studies largely failed to find the postulated benefits of antioxidants?—was addressed by **Jeffrey Blumberg** (Tufts). Antioxidants may merely serve as markers for other healthful compounds identified in observational studies or may have real health benefits that have not been discovered in randomized clinical trials (RCT) because of methodological problems concerning dose, duration, form, combination, timing of the intervention, genetic polymorphisms, or inadequate follow-up. Most RCTs have not determined plasma levels of antioxidants or levels of oxidative stress in participants. Despite these limitations, many studies have reported benefits for antioxidants, such as reduced risks for stroke, cancer, cognitive decline, and death from heart disease.

## Epigenetics, Diet, and Disease

*Chaired by Rod Dashwood (LPI) and Sharon Ross (National Cancer Institute)*

• **Rod Dashwood** (LPI) discussed the anticancer mechanisms of compounds in garlic (the metabolite allyl mercaptan) and broccoli sprouts (sulforaphane). Both compounds act as histone deacetylase (HDAC) inhibitors. When HDAC activity is inhibited, tumor suppressor genes are turned on to block tumor growth. Allyl mercaptan and sulforaphane caused dose-dependent cell-cycle arrest and apoptosis (programmed cell death) in cancer cells in culture, and sulforaphane inhibited HDAC activity in mice, as did allyl mercaptan, and in human blood cells. These are examples of epigenetics: changes in gene activity without changes in the underlying DNA. • **Irfan Rahman** (University of Rochester) explained how curcumin, a polyphenol from the spice turmeric, and resveratrol from red wine attenuate inflammation in the lung caused by oxidative stress. Curcumin and resveratrol affect cell-signaling pathways involved in chronic inflammation associated with asthma and chronic obstructive pulmonary disease (COPD) but have low bioavailability. • Using sheep as a model, **Lorraine Young** (University of Nottingham) investigated the effect of epigenetic modifications in the



*Diet and Optimum Health attendees*

maternal diet on offspring. Sheep were fed methyl-deficient diets prior to and shortly after conception, resulting in abnormal fetal development and predisposition to disease in offspring. Sheep eggs cultivated *in vitro* and then implanted were vulnerable to the chemical constituency of the culture media, with some lambs exhibiting abnormally large size. These effects were due to changes in DNA methylation (the replacement of a hydrogen atom with a hydrocarbon group, or CH<sub>3</sub>). Studies using human stem cells found that methylation affected differentiation, apoptosis, and proliferation, with ramifications for the risk for adult diseases.

## The Public Lecture

On Saturday morning in a free lecture open to the public, **Marion Nestle** (New York University) discussed the complex factors that influence what and how Americans eat. She traced many of the problems affecting our diet and obesity rates to government policies that encourage the overproduction of certain foods through subsidies and to corporate practices like the shareholder value movement of the 1980s that require agricultural and food companies to generate much higher financial returns more frequently, resulting in more food availability to the consumer. The ubiquitous “buffet syndrome” creates more opportunities for people to eat—at low cost—more non-nutritious food more often and in more places than ever before. The food caloric availability has risen from about 3,200 calories per person per day in the early 1980s to 3,900 calories per person per day now.

Changes in labeling laws allowed companies to make health claims on processed foods like cereal and also contributed to the development of functional foods, which is a big growth area. Corporate marketing practices foster brand loyalty and develop kids’ foods, resulting in the “pester factor” that causes parents to acquiesce to pestering and buy certain advertised products.

Dr. Nestle also addressed food safety, which has been an alarming issue in recent years. Spinach, tomatoes, peanut butter, pistachios, and meats have all been affected by bacterial contamination and recalls. Intentional adulteration of food produced in China with melamine to mislead about protein content caused widespread health problems for people and pets. Assurance of food safety is problematic because of our global food supply, lack of adequate testing, and oversight by multiple, competing agencies. For example, the USDA is responsible for meat and poultry safety, but the FDA has responsibility for other foods. One new proposal calls for a single agency to review risk-based food safety.

Dr. Nestle also commented on food as a social movement, illustrated by the popularity of local farmers’ markets, popular books on eating, and the humane production of animals. The “real foods movement” that originated mainly in Berkeley, California, in the 1970s emphasizes minimal processing, natural nutrients, no additives, and no hormones or antibiotics used in the production of

meat, poultry, and dairy products. Dr. Nestle distinguished between personal responsibilities: eat food, not products; eat smaller portions; support local farmers and grow and eat food at home; and educate children about a healthful diet, and social responsibilities: review marketing of food in schools to children, insist on government oversight of



*Dr. Joe Beckman (right) presents the LPI Prize for Health Research to Dr. Michael Holick (see article on p. 10)*



*Alexander Michels, Heather Kuiper and Mansi Parasramka*

food safety, and reduce government corruption in which campaign financing inhibits reasonable corporate regulation. Above all, Dr. Nestle encouraged people to eat less, consume more fruit and vegetables, move more, and enjoy eating! **LPI**





## Zinc is Crucial for DNA Integrity and Prostate Health

Yang Song, Ph.D.  
LPI Graduate Fellow

**B**efore starting my doctoral studies at Oregon State University, I received a bachelor of medicine degree from a medical school in China, majoring in Preventive Medicine. I interned for two years in Beijing General Railway Hospital and also worked in the China Disease Control and Prevention (China's CDC) for one year. These experiences made me think that the best way to improve human health is to develop strategies to *prevent* diseases. Moreover, chronic diseases like cancer and cardiovascular diseases cannot be completely treated, whereas dietary intervention strategies can effectively decrease disease incidence. Fascinated by the potent effects of various foods and nutrients in disease prevention, I decided to pursue a Ph.D. degree in the field of nutrition. Five years ago, I left Beijing and came to Oregon State University to become a graduate student in the Linus Pauling Institute.

My general research interest is nutrition and cancer; specifically, the foods or nutrients that can be used as chemoprevention agents. How do eating habits influence the risk of cancer? What micronutrients in the diet could effectively prevent cancer? How do these micronutrients work? Could they be provided as supplements to prevent cancer? These are the questions that I wanted to answer.

My research in Dr. Emily Ho's laboratory is focused on one essential micronutrient, zinc, which is present in virtually all cells. Like other essential minerals, including iron and copper, zinc is bound to many different proteins and is required for numerous cell and physiological functions, such as growth and development, immune response, wound healing, neurological function, and reproduction. Since zinc itself does not have redox (reduction-oxidation) activity, it serves as a good cofactor for a large group of proteins. The primary functions of zinc in biological systems can be categorized as catalytic, structural, and regulatory. For instance, zinc is required for the formation and stabilization of protein structure and is essential for the activity of more than 300 enzymes. Recent research suggests that zinc also serves as a regulator of activities of numerous redox-sensitive signaling molecules and transcription factors by modulating intracellular redox status.

### Human zinc deficiency

Human zinc deficiency was first described in Iran in the early 1960s by Prasad and colleagues. They observed that zinc-deficient patients displayed symptoms of "severe growth retardation, anemia, hypogonadism, hepatosplenomegaly, rough and dry skin, mental lethargy and geophagia." Human zinc deficiency is clinically categorized as either marginal or severe, based on the severity of the symptoms. Marginal zinc deficiency is one of the most prevalent mineral deficiencies in the U.S. and is usually

caused by inadequate zinc intake. Marginally zinc-deficient patients display numerous non-specific symptoms, such as growth retardation, skin changes, vulnerability to infection, and delayed wound healing. On the other hand, severe zinc deficiency is rare in developed countries. It's usually caused by inborn defects of zinc absorption or secondary factors, including liver disease, chronic renal disease, sickle-cell disease, or high intakes of unleavened flour. The affected patients suffer from severe dysfunctions of the central nervous, immune, reproductive, epidermal, and skeletal systems.

According to the International Zinc Nutrition Consultative Group, approximately two billion people worldwide are at risk for zinc deficiency. Although the true prevalence of zinc deficiency is difficult to evaluate due to the lack of sensitive and specific biomarkers, the actual prevalence has been confirmed by several zinc supplementation trials in healthy infants, toddlers, and preschool children. These trials observed improved neuro-physiologic performance, positive growth response, and significantly reduced mortality and morbidity in the zinc-supplemented populations. The prevalence of zinc deficiency is much higher in developing countries than in developed countries because of the limited source of zinc-abundant foods, such as red meat and seafood. However, the prevalence of marginal zinc deficiency in developed countries may still be high. Data from National Health and Nutrition Examination Survey (NHANES 2001-2002) supported the same conclusion, finding that approximately 12% of the population does not consume the Estimated Average Requirement for zinc and could be at risk for marginal zinc deficiency. Moreover, the prevalence of zinc deficiency is even higher in certain populations, such as children, women, and elderly people, because of the high need for zinc or reduced zinc absorption.

### Zinc deficiency and cancer

The connection between zinc deficiency and cancer has been suggested by several large epidemiological studies. These observational cohort studies found that low zinc status is associated with increased cancer incidence. Zinc deficiency impairs DNA integrity and, thus, increases the cell susceptibility to abnormal growth.

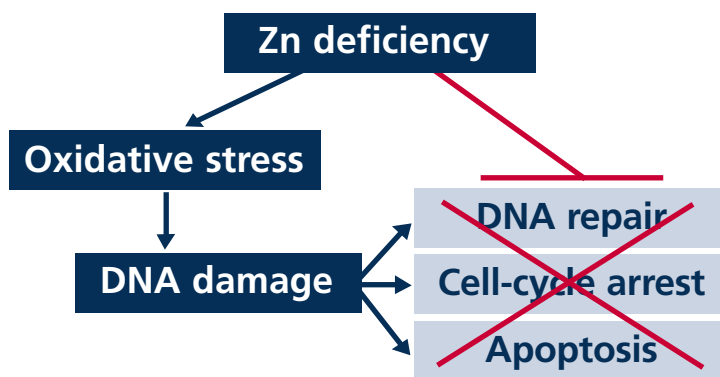
DNA damage can be caused by numerous endogenous and exogenous agents, such as ionizing radiation, ultra-violet light, reactive oxygen species, ethidium bromide, and food-borne carcinogens (polycyclic aromatic hydrocarbons and heterocyclic amines). In addition, deficiencies of micronutrients like vitamin C, vitamin E, iron, and zinc may damage DNA through breaks or oxidative modifications. Accumulation of DNA mutations is an essential event in cancer initiation, promotion, and progression. DNA damage initiates cells and may cause the progression to cancer. During the promotion stage, initiated cells start to grow and differentiate rapidly and accumulate DNA mutations, resulting in changes in cell behavior. During the progression stage, cancer cells grow aggressively, resulting in the appearance of a small tumor.

Previous *in vitro* studies in our lab have shown that zinc depletion in cells increased DNA damage. However, the effects of zinc deficiency on DNA integrity

in cells in the body are still unclear. Thus, one focus of my research has been to explore the effects of zinc depletion on DNA integrity *in vivo* and to understand the mechanisms of these effects.

Our studies confirm that dietary zinc depletion impairs DNA integrity *in vivo*. We found that both marginal and severe zinc deficiencies are capable of increasing DNA strand breaks in the peripheral blood cells of rats, but this damage was reversible and could be reduced to normal levels by zinc repletion. We also observed similar effects in human subjects with marginal zinc depletion and repletion, indicating that zinc is also essential for maintaining DNA integrity in humans.

At least two mechanisms are likely involved in the effect of zinc deficiency on DNA damage (see the figure below). First, zinc deficiency compromises the functions of the zinc-containing antioxidant enzyme, copper-zinc superoxide dismutase, and increases oxidative stress. Increased oxidative stress causes oxidative DNA damage directly. Second, zinc deficiency impairs DNA repair functions by interfering with the activities and expression of DNA repair proteins. Altogether, zinc-deficient cells take a double hit—DNA damage is increased and the ability to repair that damage is compromised.



Possible mechanism by which zinc deficiency causes DNA damage. On the one hand, zinc deficiency increases oxidative stress, which can directly generate DNA damage. On the other hand, zinc deficiency interferes with the cell functions to repair DNA damage or get rid of the damaged cell.

## Zinc and prostate cancer

Zinc has a very special connection with prostate cancer. The prostate has the highest concentration of zinc of any soft tissue in the body. Zinc concentrations in malignant prostate tissue are only about 10-25% of those in healthy prostates, suggesting that high zinc concentrations may be required for the maintenance of prostate health. However, the specific functions of zinc in the prostate and the mechanisms by which zinc maintains prostate health are still unclear.

We found that marginal zinc deficiency alone did not increase oxidative DNA damage in the prostates of rats. However, a combination of both marginal zinc deficiency and high intensity chronic exercise, which produces oxidative stress, markedly increased oxidative DNA damage in the prostate. Our study suggests that marginal

zinc deficiency sensitizes the prostate to exogenous oxidative stress. Moreover, marginal zinc depletion decreases zinc concentrations and affects DNA integrity only in the dorsolateral—not ventral—lobes of the prostate. About 70% of human prostate cancers are found in the human prostate area equivalent to the rat dorsolateral lobe. These data suggest that the prostate dorsolateral lobe—the area of the prostate most susceptible to cancer—is more sensitive to marginal zinc deficiency than the ventral lobe. Overall, our findings demonstrate the importance of maintaining good zinc status for good health in physically active people.

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## Oxygen Club of California 2010 World Congress

The biennial meeting of the Oxygen Club of California on *Oxidants and Antioxidants in Biology: Translational Redox Science*, co-sponsored by the Linus Pauling Institute, will be held on March 17-20, 2010, in Santa Barbara, California. The meeting will feature keynote lectures by Johannes von Lintig (the Norman Krinsky Memorial Lecture) and Nobel Prize winners Harald zur Hausen and Luc Montagnier, as well as scientific sessions on:

- Wound Healing
- Redox Signaling and Inflammation
- Cardiovascular Disease
- Translational Science by Micronutrients
  - Carotenoids and Vitamin A
  - Coenzyme Q
  - Polyphenols and Lipoic Acid
- Redox Imaging
- Aging

Forty-one scientists are scheduled to give presentations. Additionally, scientific posters will be presented, and both young and established investigators will be recognized and honored with awards, including the Oxygen Club of California Science Prize, the Science and Humanity Award, and the Young Investigator Awards. For more information, please visit [www.oxyclubcalifornia.org](http://www.oxyclubcalifornia.org).

# Recent Publications by LPI Scientists

*Selected summaries by Stephen Lawson*

*A comprehensive list of all LPI scientific papers published from 2007 to 2009 will be posted online as a supplement to this article. Summaries of some representative papers appear below. LPI scientists are identified in **boldface**.*

## Cancer Chemoprotection

**CARTER O, DASHWOOD RH, WANG R, DASHWOOD WM, ORNER GA, FISCHER KA, LOHR CV, PEREIRA CB, BAILEY GS, and WILLIAMS DE.** Comparison of white tea, green tea, epigallocatechin-3-gallate, and caffeine as inhibitors of PhIP-induced colonic aberrant crypts. *Nutr. Cancer* 58:60-65, 2007

In previous studies, the authors found that white tea—the least processed type of tea—inhibited the formation of lesions in rat colons induced by exposure to heterocyclic amines, frequently called “cooked-meat mutagens” because they are formed in proteinaceous food grilled or fried at high temperatures. In the present study, the authors fed white tea or green tea to rats either before and during or after exposure to a heterocyclic amine. While both teas induced liver enzymes that metabolize and aid in the excretion of the mutagen, white tea afforded the greatest protection against colonic lesions, especially when given after exposure to the mutagen.

**SIMONICH MT, EGNER P, ROEBUCK BD, ORNER G, JUBERT C, PEREIRA C, GROOPMAN JD, KENSLER TW, DASHWOOD RH, WILLIAMS D, and BAILEY GS.** Natural chlorophyll inhibits aflatoxin B<sub>1</sub> induced multi-organ carcinogenesis in the rat. *Carcinogenesis* 28:1294-1302, 2007

Prolonged exposure to aflatoxin B<sub>1</sub>, a carcinogen produced by mold growing on damp grains, causes liver cancer in many people in Asia and Africa. The authors fed chlorophyll—the green pigment in plants—or its derivative, chlorophyllin, to rats treated with aflatoxin B<sub>1</sub>. Both compounds very substantially reduced DNA damage, a surrogate for liver and colon cancer, with chlorophyll slightly more effective than chlorophyllin. Chlorophyll and chlorophyllin bind to aflatoxin B<sub>1</sub> in the gut, preventing its entry into the bloodstream and delivery to target organs.

**CASTRO DJ, YU Z, LÖHR CV, PEREIRA CB, GIOVANNI J, FISCHER KA, ORNER GA, DASHWOOD RH, and WILLIAMS DE.** Chemoprevention of dibenzo[a,l]pyrene transplacental carcinogenesis in mice born to mothers administered green tea: primary role of caffeine. *Carcinogenesis* 29:1581-1586, 2008

The authors previously reported that feeding indole-3-carbinol—a phytochemical in cruciferous vegetables—to pregnant mice exposed to a carcinogenic polycyclic aromatic hydrocarbon called dibenzo[a,l]pyrene (DBP) protected the offspring from mortality due to lymphoma and also reduced lung tumor multiplicity. Polycyclic aromatic hydrocarbons are prevalent environmental carcinogens formed by the combustion of organic material. In the present study, the authors fed green tea, decaffeinated green tea, caffeine, or epigallocatechin-

3-gallate (EGCG) from green tea to pregnant and nursing mice exposed to DBP. Only green tea and caffeine significantly protected the offspring from mortality due to lymphoma. All treatments, especially EGCG, reduced tumor multiplicity. The results show that transplacental carcinogenesis can be inhibited by the maternal consumption of green tea.

**CLARKE JD, DASHWOOD RH, and HO E.** Multi-targeted prevention of cancer by sulforaphane. *Cancer Lett.* 269:291-304, 2008

Isothiocyanates, including sulforaphane and indole-3-carbinol (I3C), are phytochemicals derived from glucosinolates in cruciferous vegetables that help protect against cancer. When cruciferous vegetables like broccoli are consumed, myrosinase enzymes in the plant and gut microbes convert the parent compounds into sulforaphane and I3C. Sulforaphane has been shown to induce Phase 1 and 2 enzymes in the liver that metabolize, detoxify, and help excrete carcinogens. New cancer chemoprotective functions for sulforaphane have been described, including cell-cycle arrest, apoptosis (programmed cell death), and histone deacetylase inhibition. When histone deacetylase is inhibited, tumor suppressor genes are turned on and cell-cycle arrest and apoptosis occur, leading to the selective death of cancer cells. Clinical studies indicate that sulforaphane is absorbed into the bloodstream, accumulates in tissues, and appears to be safe and well tolerated.

**FREI B and LAWSON S.** Vitamin C and cancer revisited. *Proc. Natl. Acad. Sci. U.S.A.* 105:11,037-11,038, 2008

The use of vitamin C to treat cancer has been controversial for many decades. In this commentary, the authors review the early work of Linus Pauling and Ewan Cameron, who treated terminal cancer patients with high-dose intravenous and oral vitamin C, as well as recent mechanistic research. Cameron and Pauling reported that many patients derived benefit from vitamin C, ranging from an increased sense of well-being and prolonged survival to complete regression. In two clinical trials, the Mayo Clinic failed to find any benefit for vitamin C; however, vitamin C was given only orally. Recent work by Mark Levine at the National Institutes of Health has elucidated how very high concentrations of vitamin C attained only by intravenous administration generate hydrogen peroxide around cancer cells that then kills them. This mechanistic explanation should stimulate better-designed clinical trials using intravenous vitamin C.

**NIAN H, DELAGE B, PINTO JT, and DASHWOOD RH.** Allyl mercaptan, a garlic-derived organosulfur compound, inhibits histone deacetylase and enhances Sp3 binding on the P21WAF1 promoter. *Carcinogenesis* 29:1816-1824, 2008

The authors screened several organosulfur compounds derived from garlic for their potential to inhibit histone



deacetylase. Such inhibition turns on tumor suppressor genes and leads to cell cycle arrest, growth inhibition, and apoptosis in cancer cells. Allyl mercaptan, the most potent histone deacetylase inhibitor, arrested the cell cycle of human colon cancer cells, resulting in growth inhibition.

**SIMONICH MT, MCQUISTAN T, JUBERT C, PEREIRA C, HENDRICKS JD, SCHIMERLIK M, ZHU B, DASHWOOD RH, WILLIAMS DE, and BAILEY GS.** Low-dose dietary chlorophyll inhibits multi-organ carcinogenesis in the rainbow trout. *Food Chem. Toxicol.* 46:1014-1024, 2008

The authors fed chlorophyll or its derivative chlorophyllin to trout treated with the environmental carcinogen dibenzo[a,l]pyrene. Chlorophyll or chlorophyllin were about equally effective in reducing the incidence of liver and stomach cancer by about half. Chlorophyllin given after exposure to the carcinogen slightly increased the incidence of cancer, although in other studies chlorophyll did not promote tumors post-initiation. Both compounds probably work by binding to the carcinogen and preventing its uptake into tissues. The chemoprotective amount of chlorophyll is within the range found in typically consumed portions of spinach.

**CHUNG WG, MIRANDA CL, STEVENS JF, and MAIER CS.** Hop proanthocyanidins induce apoptosis, protein carbonylation, and cytoskeleton disorganization in human colorectal adenocarcinoma cells via reactive oxygen species. *Food Chem. Toxicol.* 47:827-836, 2009

Proanthocyanidins are polyphenols found in many fruits, vegetables, beverages, cocoa, and hops used in the production of beer. The authors tested the anticancer effect of proanthocyanidins from hops in human colon cancer cells, finding that the proanthocyanidins killed the cells in a dose-dependent manner by apoptosis (programmed cell death), cytoskeleton derangement, oxidative damage to proteins, and induction of the formation of hydrogen peroxide. Although the absorption of proanthocyanidins into the bloodstream may be very limited, there is evidence that high local concentrations in the colon can be attained. The anticancer concentrations cannot be achieved by drinking beer.

**NIAN H, BISSON WH, DASHWOOD WM, PINTO JT, and DASHWOOD RH.**  $\alpha$ -Keto acid metabolites of organoselenium compounds inhibit histone deacetylase activity in human colon cancer cells. *Carcinogenesis* 30:1416-1423, 2009

Low levels of selenium have been associated with an increased risk for cancer in several studies, but supplementation trials have yielded conflicting results. In this mechanistic study, the authors tested two organic forms of selenium (methylselenocysteine and selenomethionine) and their liver metabolites ( $\beta$ -methylselenopyruvate and  $\alpha$ -keto- $\gamma$ -methylselenobutyrate, respectively) for their ability to inhibit histone deacetylase in human colon cancer cells. Inhibition of histone deacetylase results in cell-cycle arrest and cell death. While the parent compounds did not appreciably affect histone deacetylase activity, the metabolites were inhibitory in a dose-dependent manner, resulting in apoptosis (programmed cell death).

**SONG Y, CHUNG CS, BRUNO RS, TRABER MG, BROWN KH, KING JC, and HO E.** Dietary zinc restriction and repletion affects DNA integrity in healthy men. *Am. J. Clin. Nutr.* 90:321-328, 2009

It is estimated that about two billion people worldwide have inadequate zinc status, although specific and sensitive biomarkers for zinc deficiency have not been validated. In this study, the authors dietarily depleted nine healthy men of zinc for about six weeks, then repleted them with 11-20 mg/day of zinc for about four weeks. During the zinc-depletion part of the study, DNA damage in blood cells increased, and zinc repletion reversed DNA damage. Levels of F<sub>2</sub>-isoprostanes—markers of lipid peroxidation—were unaffected by zinc status throughout the study. The authors conclude that adequate zinc status maintains DNA integrity, which may reduce the risk for cancer.



**YAN M, HARDIN K, and HO E.** Differential response to zinc-induced apoptosis in benign prostate hyperplasia and prostate cancer cells. *J. Nutr. Biochem.* 2009 [Epub ahead of print].

The prostate has the highest concentration of zinc of all soft tissues in the body, but malignant prostate cells have low levels of zinc. The authors investigated the effect of zinc on prostate cancer cells and benign prostate hyperplasia (BPH) cells *in vitro*. Zinc added to the culture media at concentrations higher than those found in plasma but lower than levels found in the prostate induced apoptosis (programmed cell death) in BPH cells. Higher concentrations of zinc were required to kill the prostate cancer cells. Zinc affected molecular pathways in the BPH and prostate cancer cells differently. Overall, it appears that zinc helps prevent prostate cancer, including in men with BPH, but the therapeutic value in prostate cancer may be limited.

## Cardiovascular and Metabolic Diseases

**BRUNO RS, SONG Y, LEONARD SW, MUSTACICH DJ, TAYLOR AW, TRABER MG, and HO E.** Dietary zinc restriction in rats alters antioxidant status and increases plasma F<sub>2</sub> isoprostanes. *J. Nutr. Biochem.* 18:509-518, 2007

In this study, the authors report that dietary restriction of zinc in rats resulted in an increase in oxidative stress as

*continued on page 11*



# The Linus Pauling Institute Prize for Health Research

The 2009 LPI Prize for Health Research, consisting of a medal and \$50,000, was awarded at the Diet and Optimum Health Conference on May 15<sup>th</sup> to

Michael Holick, M.D., Ph.D. Dr. Holick is Professor of Medicine, Physiology, and Biophysics at Boston University School of Medicine. He earned his B.S. degree in biology at Seton Hall University and his Ph.D. and M.D. degrees at the University of Wisconsin-Madison. He completed his internship and residency at Massachusetts General Hospital in Boston. He has held academic positions at Harvard, MIT, and Tufts, and been at Boston University since 1987, conducting research and caring for patients. Dr. Holick has published over 320 scientific papers and over 209 reviews and book chapters and has written or edited nine books. He joins Drs. Bruce Ames (2001), Walter Willett (2003), Paul Talalay (2005), and Mark Levine (2007) as recipients of the LPI Prize.

Dr. Holick's entertaining plenary lecture consisted of hundreds of slides illustrating, often very humorously, the importance of vitamin D in health. Perhaps more than any other individual, Dr. Holick has championed the benefits of vitamin D above and beyond its vitamin role in preventing rickets. A number of nominators compared Dr. Holick's advocacy of vitamin D to Linus Pauling's advocacy of vitamin C. Both vitamins have been shown to carry out critical functions unrelated to their vitamin roles in preventing deficiency diseases; the intake or blood/tissue status of each is marginal in many people in the United States; and both Drs. Holick and Pauling faced tremendous skepticism, even from their colleagues, about their claims and recommendations. As one nominator wrote, "I well remember Linus Pauling standing up to criticism and skepticism, a trait of Holick as well." Another nomination letter catalogued Dr. Holick's original contributions to vitamin D science—he was the first to isolate and identify 25-hydroxyvitamin D<sub>3</sub> in human blood; the first, with Hector DeLuca, to chemically synthesize 1,25-dihydroxyvitamin D<sub>3</sub>; the first to identify how vitamin D is produced in the skin; and the first to demonstrate that vitamin D is safe and effective for treating psoriasis. He also demonstrated how skin pigmentation, the use of sunscreens, and latitude affect vitamin D synthesis, concluding that supplemental vitamin D is necessary for most people living in northern latitudes or shielded from the sun in the summer. Indeed, his studies stimulated general acceptance among health professionals for a change in the threshold for vitamin D deficiency or sufficiency from <10 ng/ml in blood to <30 ng/ml. Blood levels of vitamin D can be raised 1 ng/ml by every 100 IU of ingested vitamin D.

Dr. Holick stated that vitamin D deficiency is the most common micronutrient deficiency in the world, occurring in over 50% of the people in the U.S., Europe, China, India, and elsewhere. Vitamin D deficiency is linked to an increased risk for cancer, diabetes, autoimmune diseases, bone disease, multiple sclerosis, rheumatoid arthritis, infectious illnesses, upper respiratory

tract infections, tuberculosis, and the risk of dying from cancer or heart disease. Dr. Holick noted that the highest intake of vitamin D correlates with the least incidence of cancer. Antimicrobial activities of vitamin D may also improve resistance to influenza.

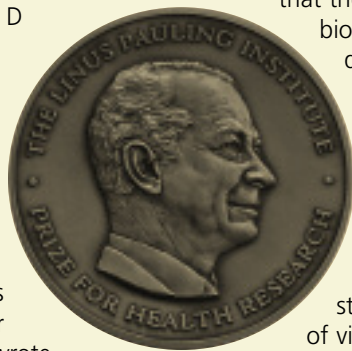
Upon exposure to sunlight, previtamin D<sub>3</sub> is formed in the skin from cholesterol and, after another chemical modification, enters the circulation. In the liver, vitamin D<sub>3</sub> (cholecalciferol) is converted to 25-hydroxyvitamin D (calcidiol), which is then hydroxylated in the kidney to its biologically active form, 1,25-dihydroxyvitamin D (calcitriol). Both vitamin D<sub>2</sub> (ergocalciferol, made in plants) and D<sub>3</sub> raise blood levels of 25-hydroxyvitamin D. Calcidiol persists in the blood for several weeks, whereas calcitriol disappears after hours. To determine vitamin D status, Dr. Holick noted that laboratory tests for the major circulating form of vitamin D—25-hydroxyvitamin D—not 1,25-dihydroxyvitamin D, which is the test often ordered by physicians, should be performed. Dr. Holick related the story of his own work in identifying vitamin D<sub>3</sub> in human blood and its synthesis and the isolation of vitamin D from chicken organs, a particularly unpleasant task.

Almost all cells in the body have vitamin D receptors, indicating that the vitamin plays a very important role in cellular biology. Even reptiles in captivity become vitamin D deficient without sun or UV light exposure.

Historically, vitamin D has been recognized for its requirement for normal bone growth. There are still a few cases of rickets in Boston, owing to the lack of vitamin D in breast milk. To achieve satisfactory vitamin D status in infants, Dr. Holick recommends an intake of 4,000 IU/day for lactating mothers to ensure adequate vitamin D status in nursing infants. Compounding the problem of vitamin D insufficiency, virtually no vitamin D is synthesized in the skin in latitudes north of Atlanta, Georgia, in the winter. In the summer, use of an SPF-30 sunscreen decreases endogenous synthesis of vitamin D by 99%. Recognizing the increased risk for skin cancer with excessive sun exposure, Dr. Holick advises sun exposure of arms and legs for 5-15 minutes about two or three times per week in northern latitudes like Boston. He suggests about 15-30 minutes of sun exposure two or three times per week for the elderly, about two to three times more for the obese, and about five to ten times more for people with dark skin. Generally, Dr. Holick recommends at least 400 IU/day of vitamin D<sub>2</sub> or D<sub>3</sub> for children and 1,400-2,000 IU/day for adolescents and adults. Toxicity is very rare and doesn't occur until at least 10,000 IU of vitamin D have been consumed daily for at least five months.

The LPI Prize for Health Research recognizes and honors Dr. Holick's fundamental and provocative contributions to our knowledge about the critical importance of vitamin D in human health.

A brief interview with Dr. Holick is posted on the LPI Web site as a podcast (<http://lpi.oregonstate.edu/podcast>). To learn more about vitamin D, please visit LPI's Micronutrient Information Center (<http://lpi.oregonstate.edu/infocenter/vitamins/vitaminD>).





measured by F<sub>2</sub>-isoprostanes, which are formed from the oxidation of arachidonic acid, a polyunsaturated fat. The increased oxidative stress was associated with declines in levels of plasma uric acid and vitamin E in the liver, possibly because these antioxidants were used up by increased levels of oxidants. Plasma vitamin C was unaffected. Zinc appears to function as an antioxidant indirectly by enhancing the activity of liver enzymes that metabolize arachidonic acid and by maintaining the activity of glutathione, an important endogenous antioxidant. As many as 12% of Americans may be zinc deficient, thus compromising their antioxidant status.

**LEONARD SW, JOSS JD, MUSTACICH DJ, BLATT DH, LEE YS, and TRABER MG.** Effects of vitamin E on cholesterol levels of hypercholesterolemic patients receiving statins. *Am. J. Health. Syst. Pharm.* **64**:2257-2266, 2007

Based on studies of increased xenobiotic (drug) metabolism in mice fed vitamin E, the authors tested the hypothesis that vitamin E supplementation in hypercholesterolemic humans taking lovastatin or simvastatin to lower cholesterol would negate the beneficial effect of the statins. Surprisingly, vitamin E (400 IU/day for eight weeks) did not affect total or low-density cholesterol levels but did slightly lower HDL cholesterol levels.

**TRABER MG and ATKINSON J.** Vitamin E, antioxidant and nothing more. *Free Radic. Biol. Med.* **43**:4-15, 2007

The authors suggest that the sole role of vitamin E in humans is to act as a scavenger of peroxy radicals, thus inhibiting lipid peroxidation. Preventing lipid peroxidation in membranes preserves their vital biological functions. The alpha-tocopherol transfer protein (alpha-TTP) recognizes only alpha-tocopherol (natural vitamin E) for distribution to tissues; thus, the vitamin E requirement for humans is based on alpha-tocopherol. Defects in alpha-TTP in humans cause severe vitamin E deficiency, leading to ataxia and neuropathy. The authors propose that the regulation of molecular signaling pathways often attributed to vitamin E is instead dependent on oxidative stress.

**DEVARAJ S, LEONARD S, TRABER MG, and JIALAL I.** Gamma-tocopherol supplementation alone and in combination with alpha-tocopherol alters biomarkers of oxidative stress and inflammation in subjects with metabolic syndrome. *Free Radic. Biol. Med.* **44**:1203-1208, 2008

Metabolic syndrome increases the risk for diabetes and heart disease, probably due to increased oxidative stress and inflammation. The RDA for vitamin E is based on alpha-tocopherol, or natural vitamin E, an important fat-soluble antioxidant. Gamma-tocopherol, the most abundant form of vitamin E in the American diet, is also an antioxidant that prevents nitrative damage to proteins. The authors conducted a trial with 80 men and women with metabolic syndrome randomized to receive either 800 mg/day of alpha-tocopherol, 800 mg/day of gamma-tocopherol, 800 mg/day of each, or placebo for six weeks. The combination of alpha- and gamma-tocopherol significantly decreased levels of C-reactive protein, a marker of inflammation. Oxidative stress, as measured by lipid peroxides in blood, was diminished by either form of vitamin E and both forms in combination. Nitrative stress,

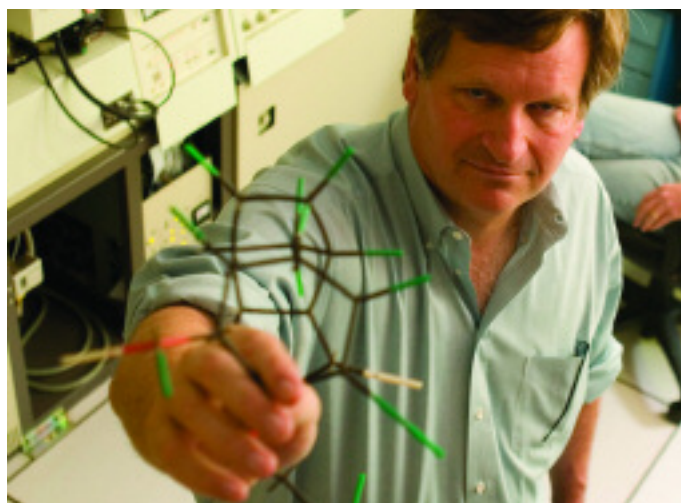
assessed by urinary nitrotyrosine, was reduced only by gamma-tocopherol alone or in combination.

**JUMP DB.** N-3 polyunsaturated fatty acid regulation of hepatic gene transcription. *Curr. Opin. Lipidol.* **19**:242-247, 2008

In this review, the author notes that omega-3 polyunsaturated fats, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) commonly found in fish, regulate genes in the liver that control carbohydrate and fat metabolism. For example, omega-3 fatty acids increase the oxidation of fats while inhibiting their synthesis and storage. Omega-3 fats affect glucose utilization and do not contribute to insulin resistance. Therefore, they may help prevent metabolic syndrome.

**TRABER MG, FREI B, and BECKMAN JS.** Vitamin E revisited: do new data validate benefits for chronic disease prevention? *Curr. Opin. Lipidol.* **19**:30-38, 2008

Vitamin E prevents the propagation of lipid peroxidation and inhibits platelet aggregation, although results of studies investigating the role of vitamin E in disease prevention have been conflicting. In this review, the authors suggest that several problems have plagued the observational and clinical studies: 1) plasma levels of vitamin E (influenced by whether fat-soluble supplements are taken with food) were seldom measured, and 2) levels of oxidative stress in



subjects were rarely determined to identify the cohort that might respond to antioxidant supplementation. Nevertheless, there are several good studies showing health benefits for vitamin E in protecting neurological function and decreasing the risk of mortality from heart disease or cancer.

**ZHANG W-J, BIRD K, MCMILLEN T, LEBOEUF R, HAGEN TM, and FREI B.** Dietary alpha-lipoic acid supplementation inhibits atherosclerotic lesion development in apolipoprotein E-deficient and apolipoprotein E/low-density lipoprotein receptor-deficient mice. *Circulation* **117**:421-482, 2008

Mice specially bred as a model of atherosclerosis were fed a Western-type diet containing cholesterol and saturated fat with and without alpha-lipoic acid supplements for ten weeks.

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Supplementation significantly reduced atherosclerotic lesion formation and triglyceride levels. Alpha-lipoic acid also inhibited the expression of pro-inflammatory molecules and adhesion molecules in the aorta that are associated with the development of atherosclerotic lesions.



Additionally, supplemented mice gained 40% less body weight than unsupplemented mice.

**BUTLER JA, HAGEN TM, and MOREAU R.** Lipoic acid improves hypertriglyceridemia by stimulating triacylglycerol clearance and down regulating liver triacylglycerol secretion. *Arch. Biochem. Biophys.* **485**:63-71, 2009

Elevated levels of triglycerides in blood (hypertriglyceridemia) are causally related to diabetes and heart disease. In this study, the authors fed chow

supplemented with alpha-lipoic acid for five weeks to rats bred as a model of diabetes. They found that alpha-lipoic acid inhibited the expression of genes in the liver involved in the synthesis of fatty acids and triglycerides and decreased the secretion of triglycerides from the liver. In a related experiment, they determined that intravenously administered alpha-lipoic acid stimulated clearance of triglycerides from blood. The results suggest that alpha-lipoic acid may have utility in treating hypertriglyceridemia and diabetic dyslipidemia in humans.

**MIRANDA CL, REED RL, KUIPER HC, ALBER S, and STEVENS JF.** Ascorbic acid promotes detoxification and elimination of 4-hydroxy-2(E)-nonenal in human monocytic THP-1 cells. *Chem. Res. Toxicol.* **22**:863-874, 2009

Reactive oxygen species can induce the formation of lipid hydroperoxides like 4-hydroxy-2(E)-nonenal, or HNE, which can, in turn, generate oxidants and protein carbonyls that damage cells, leading to cell death. The authors report that vitamin C protects cells from damage by HNE by increasing the formation of glutathione conjugates of HNE and then clearing the conjugates from cells. Glutathione is an endogenous antioxidant that detoxifies cellular toxicants like HNE. This pathway represents yet another health-promoting mechanism of vitamin C.

**MUSTACICH DJ, GOHIL K, BRUNO RS, YAN M, LEONARD SW, HO E, CROSS CE, and TRABER MG.** Alpha-tocopherol modulates genes involved in hepatic xenobiotic pathways in mice. *J. Nutr. Biochem.* **20**:469-476, 2009

Phase 1 and 2 liver enzymes help detoxify xenobiotics, including drugs and some phytochemicals, by chemically modifying them to aid in their excretion. In this study, mice were fed large amounts of vitamin E for four months and their liver enzymes were measured. Supplemental vitamin E increased the expression of several enzymes involved in xenobiotic metabolism, suggesting that high intakes of vitamin E in humans may affect drug metabolism.

**ROBERTS LJ 2ND, TRABER MG, and FREI B.** Vitamins E and C in the prevention of cardiovascular disease and cancer in men. *Free Radic. Biol. Med.* **46**:1558, 2009

The authors wrote this letter in response to the Physicians' Health Study II, which reported that long-term use of vitamin C (500 mg daily) and vitamin E (400 IU every other day) had no effect on the incidence of heart attacks and strokes, deaths from cardiovascular disease, the risk of prostate or total cancers, or cancer mortality in a group of 14,641 U.S. male physicians in a ten-year period. The letter notes several flaws in the study, including the lack of measurements of oxidative stress or blood levels of the antioxidants. The "healthy enrollee effect" may have also lessened the chance for finding any effect, since the physicians had a lower risk for heart disease and cancer compared to non-enrollees. Furthermore, the amount and type of vitamin E may have been inadequate to substantially lower oxidative stress.

## Healthy Aging

**MILGRAM NW, ARAUJO JA, HAGEN TM, TREADWELL BV, and AMES BN.** Acetyl-L-carnitine and  $\alpha$ -lipoic acid supplementation of aged beagle dogs improves learning in two landmark discrimination tests. *FASEB J.* **21**:3756-3762, 2007

In a group of 12 old beagles (7.6-8.8 years old), six dogs supplemented twice daily with alpha-lipoic acid and acetyl-L-carnitine performed significantly better than unsupplemented dogs in sensitive tests of complex learning and spatial abilities. Similar results were previously obtained with rodents, but this was the first test with dogs. The authors propose that the results were due to improved mitochondrial activity in the brain and, possibly, enhanced synthesis of the neurotransmitter acetylcholine.

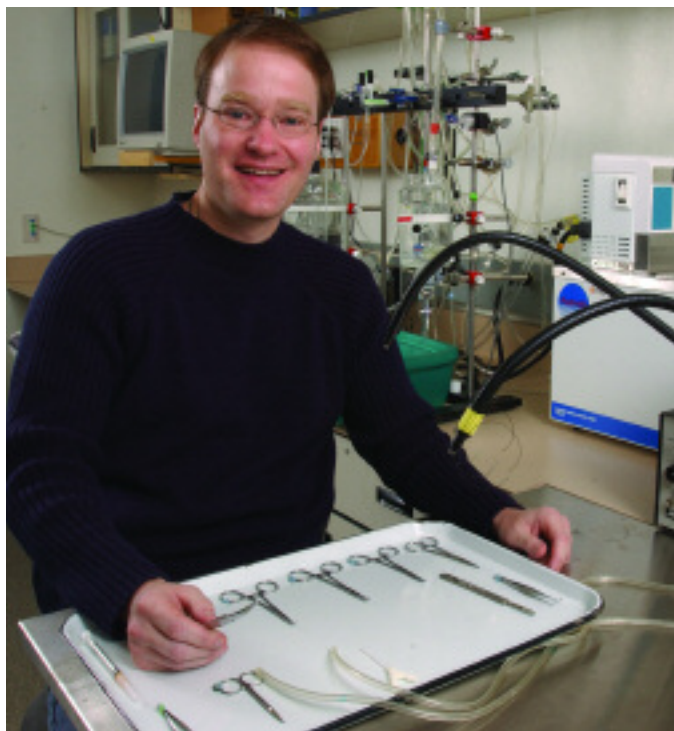
**CASSINA P, CASSINA A, PEHAR M, CASTELLANOS R, GANDELMAN M, DE LEÓN A, ROBINSON KM, MASON RP, BECKMAN JS, BARBEITO L, and RADI R.** Mitochondrial dysfunction in SOD1G93A-bearing astrocytes promotes motor neuron degeneration: prevention by mitochondrial-targeted antioxidants. *J. Neurosci.* **28**:4115-4122, 2008

Astrocytes provide structural and metabolic support for motor neurons. Mutations in the endogenous antioxidant copper-zinc superoxide dismutase (SOD), resulting in its loss of zinc, cause the altered SOD to help generate damaging reactive oxygen and nitrogen species. Astrocytes that express the mutated SOD become highly reactive and damage motor neurons, contributing to the development of amyotrophic lateral sclerosis (ALS). In this *in vitro* study, the authors report that mitochondrial dysfunction in

astrocytes with the mutant SOD cause astrocyte activation leading to motor neuron death and that this can be prevented by incubating the astrocytes with the antioxidant ubiquinone, or coenzyme Q.

**PETERSEN SHAY K, MOREAU RF, SMITH EJ, and HAGEN TM.** Is alpha-lipoic acid a scavenger of reactive oxygen species *in vivo*? Evidence for its initiation of stress signaling pathways that promote endogenous antioxidant capacity. *IUBMB Life* 60:362-367, 2008

Acting as an antioxidant, lipoic acid scavenges reactive oxygen and nitrogen species. However, an oral dose of lipoic acid is quickly cleared from the blood, suggesting that lipoic acid does not function primarily as a physiological antioxidant. The authors review evidence that supports the role of lipoic acid as an indirect antioxidant through its induction of the endogenous antioxidant glutathione. Lipoic acid also inhibits the activation of NF- $\kappa$ B, a transcription factor that increases gene expression of pro-inflammatory cytokines. Lipoic acid improves glycemic handling and has been used in Europe to treat diabetic neuropathy for over 30 years. Lipoic acid exerts these favorable effects by altering cell-signaling pathways and by acting as a weak hormetic agent that induces stress responses. In aging studies in rats and dogs, lipoic acid has been found to improve mitochondrial function, reversing age-related declines in cognitive ability and physical activity.



**SMITH AR, VISIOLI F, FREI B, and HAGEN TM.** Lipoic acid significantly restores, in rats, the age-related decline in vasomotion. *Brit. J. Pharmacol.* 153:1615-1622, 2008

Cellular ceramides—membrane lipids composed of fatty acids and sphingosine that serve as signaling molecules—increase with age. This increase is associated with decreases in glutathione, an important endogenous antioxidant, and loss of proper arterial relaxation dependent on nitric oxide activity, leading to a “stiffening” of the arteries and impaired function. The authors found that injecting lipoic

acid into old rats increased glutathione levels, decreased ceramide levels, and reversed vasomotor decline. Since age-related vasomotor dysfunction is a feature of heart disease, lipoic acid may provide a therapeutic intervention.

**BECKMAN JS.** Understanding peroxynitrite biochemistry and its potential for treating human diseases. *Arch. Biochem. Biophys.* 484:114-116, 2009

The author and his colleagues established peroxynitrite, which is formed from reactions between the superoxide radical and nitric oxide, as a biological oxidant. Peroxynitrite can nitrate the amino acid tyrosine in proteins, leading to functional impairment. In the case of acetaminophen poisoning, the therapeutic reduction of peroxynitrite formation decreases tyrosine nitration correlated with liver damage. Additionally, motor neuron death in amyotrophic lateral sclerosis (ALS) can be initiated by peroxynitrite. Peroxynitrite chemistry is very complex, but as more is learned, the possibility of therapeutic interventions increases. For example, urate, a physiological antioxidant that causes gout in very high concentrations, blocks tyrosine nitration without scavenging peroxynitrite and is associated with a reduced risk for multiple sclerosis and Parkinson's disease.

**BOWMAN GL, DODGE H, FREI B, CALABRESE C, OKEN BS, KAYE JA, and QUINN JF.** Ascorbic acid and rates of cognitive decline in Alzheimer's disease. *J. Alzheimers Dis.* 16:93-98, 2009

Alzheimer's disease is related to oxidative damage in the brain. The authors analyzed the vitamin C content in plasma and cerebrospinal fluid (CSF) from 32 patients with mild to moderate Alzheimer's disease, finding that the highest ratio of vitamin C in CSF to vitamin C in plasma was associated with the slowest decline in cognitive function. They also reported that patients with impaired blood-brain barrier function, which provides a junction between blood and CSF—protecting the brain by permitting only small molecules to pass—exhibited lower CSF to plasma vitamin C levels. Therefore, blood-brain barrier dysfunction may accelerate decline in Alzheimer's disease.

**GOMBART AF, BHAN I, BORREGAARD N, TAMEZ H, CAMARGO JR CA, KOEFFLER HP, and THADHANI R.** Low plasma level of cathelicidin antimicrobial peptide (hCAP18) predicts increased infectious disease mortality in patients undergoing hemodialysis. *Clin. Infect. Dis.* 48:418-424, 2009

Death from infections is the second most common cause of mortality among patients with end-stage renal disease undergoing hemodialysis. In this study, the authors analyzed the levels of cathelicidin, an antimicrobial protein whose synthesis is dependent on vitamin D, in 279 renal patients undergoing hemodialysis. They found that those patients with the lowest levels of cathelicidin in plasma had a two-fold increased risk of death from infections compared to those patients with the highest levels. Since the development of microbial resistance to antimicrobial peptides is rare, strategies that enhance cathelicidin's activity, such as vitamin D supplementation, may be useful in decreasing mortality.

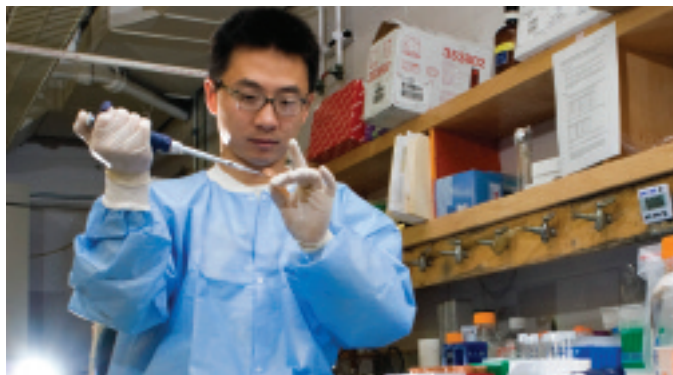
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**GOMBART AF, SAITO T, and KOEFFLER HP.** Exaptation of an ancient Alu short interspersed element provides a highly conserved vitamin D-mediated innate immune response in humans and primates. *BMC Genomics* 10:321, 2009

The authors showed that the cathelicidin antimicrobial peptide gene, controlled by vitamin D, has been evolutionarily conserved in primates for 55-60 million years. Its persistence in primates, including humans, but not in other mammals has been mediated by Alu genetic elements in so-called “junk” DNA. The authors propose that cathelicidin helps regulate the delicately balanced immune response, optimizing innate immunity while minimizing damage to the host by suppressing inflammation. The biological importance of cathelicidin activity is demonstrated by its evolutionary conservation for many millions of years.

**TRUMBULL KA and BECKMAN JS.** A role for copper in the toxicity of zinc-deficient superoxide dismutase to motor neurons in amyotrophic lateral sclerosis. *Antioxid. Redox Signal.* 11:1627-1639, 2009

In this review, the authors revisit the hypothesis that the loss of zinc from the endogenous antioxidant copper-zinc



superoxide dismutase (SOD) causes the mutant protein to become toxic to motor neurons, leading to amyotrophic lateral sclerosis, or ALS. Mutations to SOD account for 20-25% of ALS cases. Zinc is not as tightly bound to SOD as copper, and if zinc is lost, the resultant copper SOD helps to generate peroxynitrite and other radicals that kill motor neurons. Scientists have observed that SOD aggregates are associated with ALS, but the authors suggest that the protein aggregation is merely a means to remove the mutant SOD rather than a causal factor in motor neuron death. In mouse models of ALS, copper chelators and appropriate zinc supplementation delayed the progression of the disease.

LPI



Groundbreaking for the new 105,000-square-foot building to house LPI and part of the Chemistry Department occurred in late August. On September 25<sup>th</sup>, Oregon State University welcomed lead donors Tamara Valley of The Wayne and Gladys Valley Foundation and Patricia and Al Reser; Linus Pauling, Jr.; Dave Hunt, Speaker of the House of the Oregon Legislative Assembly; George Pernsteiner, Chancellor of the Oregon University System; and representatives of Andersen Construction and ZGF Architects to a ceremony celebrating the launch of building construction. Over 300 people attended the event, which featured presentations by Balz Frei of LPI; Ed Ray, President of OSU; Michael Goodwin, President and CEO of the OSU Foundation; Sherman Bloomer, Dean of the College of Science; and Kelsie Warner, an undergraduate chemistry student.

The Linus Pauling Science Center, scheduled for completion in June, 2011, will be the University's largest academic building. It will allow LPI's scientists and staff to work together in the same building for the first time since the Institute's move to Oregon in 1996 and

## The Linus Pauling Science Center Construction Launch

should foster the sort of interdisciplinary collaborations that have become so critical in research.

The building is named for OSU's most prominent alumnus. Ed Ray, President of OSU, noted that Linus Pauling “would probably qualify as any university's most distinguished alumnus.” Pauling revolutionized chemistry with his work on the chemical bond, for which he won his first Nobel Prize. He made many seminal discoveries, including a main structural theme of proteins, the alpha helix, and the molecular cause of sickle-cell anemia. He was called the major founder of the sciences of molecular biology and molecular medicine and codified orthomolecular medicine, which forms the basis for LPI's mission, in a paper published in *Science* in 1968. In the “Millennium Essay” in *Nature* in 2000, Pauling was declared one of “the great thinkers and visionaries of the millennium,” along with Galileo, Da Vinci, Newton, and Einstein.

More information about the ceremony, as well as a webcam view of the construction progress, can be found at <http://osufoundation.org/fundraisingpriorities/facilities/lpsc>. LPI





# Developments

Scott Palmer  
LPI Director of Development

## What a Great Day!

It was a glorious fall day in Corvallis—a warm, sun-drenched afternoon, with just a hint of a breeze underneath a beautiful blue sky with a handful of puffy white clouds along the top of the Coast Range to the West. But then, given the magnitude of the event that unfolded before us, how could the weather have been anything other than perfect?

On Friday, September 25<sup>th</sup>, we celebrated the construction launch of the Linus Pauling Science Center on the campus of Oregon State University. With a crowd of several hundred people at the construction site, Dr. Balz Frei began the formal program, which included comments from OSU President Edward J. Ray, OSU Foundation President Michael Goodwin, Campaign for OSU Co-Chair Patricia J. Reser, Oregon State Speaker of the House Dave Hunt, Oregon University System Chancellor George Pernsteiner, OSU student Kelsie E. Warner, and College of Science Dean Sherman Bloomer.

The Wayne and Gladys Valley Foundation of Oakland, California, and the Reser family of Beaverton, Oregon, were especially thanked for their incredibly generous gifts that provided the majority of private funds needed to secure matching funds from the State of Oregon. But the real star of the day was the big hole in the ground right in front of us; a hole from which huge upright beams of structural steel are already sprouting—solid confirmation that the dream of a state-of-the art research center to house the Linus Pauling Institute is but just two years away.

The impact of this facility on our ability to conduct innovative research will be enormous. Once completed, the Linus Pauling Science Center will fundamentally change the way we operate. For LPI, collaboration has always been critical, and we have achieved much even with scientists and labs spread across the OSU campus. Bringing all of our researchers together will create a scientific critical mass that will greatly enhance the interaction and collaborations that are such an important part of the creative process. But the building is only the beginning. To take full advantage of the opportunities and changes ahead of us, we need your help.

Recent advances in epigenetics, immunosenescence, and other fields suggest tantalizing new research paradigms that have the potential to greatly expand our knowledge about the mechanisms of aging, heart disease, and cancer. Achieving that potential will require

an innovative, multi-disciplinary approach. The Linus Pauling Institute scientists have the capacity to conduct astoundingly innovative research, but that research costs money. That's where you come in.

The unfortunate reality is that highly innovative research projects rarely get funded by the traditional funding entities. The competition for research grants from the National Institutes of Health and other major funding entities is fierce. With fewer dollars available to support research, there is a natural tendency to support more conservative research that has a high potential for success. The gifts we receive from our friends and donors provide the scientists at LPI with a significant amount of leverage to conduct initial pilot research projects that can lead to much larger grants in the future. Your support allows us to ask the question, "What if?," and then try to answer that question.

Every gift, regardless of size, is important and much appreciated. With the start of construction of our new building, there is increasing interest in the opportunity to name a room or a lab within the Linus Pauling Science Center. Naming opportunities start at \$25,000 and go up to \$500,000. For more information, please contact Kevin Heaney at (541) 737-5495 or at [kevin.heaney@oregonstate.edu](mailto:kevin.heaney@oregonstate.edu).

Another change, which is much less significant than this great project, is that the time has come for me to leave the Linus Pauling Institute and Oregon State University Foundation. Being associated with the Institute has been a wonderful experience for me. I will greatly miss working with Balz Frei and the other scientists and staff of the Institute. But most of all, I will miss spending time with the hundreds of donors and supporters, like you, whose loyalty and generosity has been so crucial to the success of the Linus Pauling Institute. It truly has been an honor to be a part of this amazing adventure. **LPI**



# Linus Pauling Institute

*Stephen Lawson, Research Newsletter Editor*

Oregon State University  
571 Weniger Hall  
Corvallis, Oregon 97331-6512

phone: 541-737-5075  
fax: 541-737-5077  
email: [lpi@oregonstate.edu](mailto:lpi@oregonstate.edu)  
Internet Web site: <http://lpi.oregonstate.edu>



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## 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.

## Micronutrient Research for Optimum Health



## *Look for these informative articles inside!*

Page 1 .....*From the Director*

Page 1 .....*LPI Diet and Optimum Health Conference*

Page 2 .... *Micronutrients and Violent Behavior*

Page 6 .... *Zinc is Crucial for DNA Integrity and Prostate Health*

Page 7 .... *Oxygen Club of California 2010 World Congress*

Page 8 .... *Recent Publications by LPI Scientists*

Page 10 .... *The Linus Pauling Institute Prize for Health Research*

Page 14 .... *The Linus Pauling Science Center Construction Launch*

Page 15 .... *Developments*

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