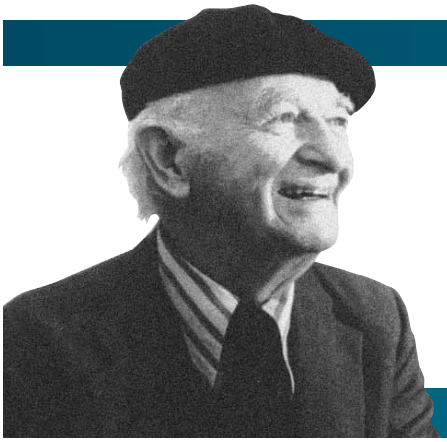
F A L L / W I N T E R 2 0 1 3



OSU Oregon State University

The Linus Pauling Institute

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

What are the most pressing health problems that will face our society in the coming decades?

How can the Linus Pauling Institute contribute to the resolution of society's most pressing health problems?

Which knowledge-based tools and skills will be necessary for LPI to successfully address our most pressing health problems in innovative ways, especially as they involve LPI's research, outreach, and communications efforts?

In 1996, a Memorandum of Understanding was signed between the Linus Pauling Institute of Science and Medicine in Palo Alto, California, and Oregon State University that established the Linus Pauling Institute on the OSU campus. Over the past 17 years, thanks to the outstanding faculty, staff, and students who joined the Institute, we have enjoyed considerable growth and remarkable successes in scientific research and many other areas, capped in 2011 by the completion of our new building, the Linus Pauling Science Center. Now that we have achieved the main goals and objectives set forth in the Memorandum of Understanding, it is time to once again carefully plan for the future.

To move LPI successfully into this next phase, we have embarked upon a process to determine our strategic direction for the next five years. Our process is designed to help us consider where we would like to be in 10 to 15 years and then determine how we can achieve our aspirations. Our strategic plan will help us build upon our strengths and enhance excellence, which includes meaningful impact to meet the health needs of our society.

Key participants in this planning project include LPI's faculty and staff, other OSU faculty and administrators, our peers and scientific colleagues at other universities, and our donors and advocates of the work we do at the

continued on page 2

The Diet and Optimum Health Conference

Stephen Lawson
LPI Administrative Officer

The seventh biennial LPI Diet and Optimum Health Conference, co-sponsored by the Oxygen Club of California, convened on the OSU campus in Corvallis from May 15-18. The conference featured 28 speakers from around the world and was organized into six sessions:

- *Omega-3 Fatty Acids in Cardiovascular and Metabolic Disease Prevention*
- *Vitamin D—Health Benefits Beyond Bone*
- *Health Effects and Mechanisms of Action of Xanthohumol*
- *Diet and Epigenetic Impacts on Disease and Aging*
- *Health Benefits of Vitamin C: Beyond Scurvy*
- *Micronutrients in Fertility and Pregnancy*

Sixty-eight posters depicting experimental projects were displayed beginning Thursday evening. An additional eight posters were selected for oral presentations on Friday afternoon.

Additionally, Saturday morning featured a public session—*Whole-food Approaches to Disease Prevention*.

The Linus Pauling Institute Prize for Health Research was presented on Friday to **Helmut Sies** of Heinrich Heine University Düsseldorf, who gave an engrossing lecture on carotenoids, flavonoids, and oxidative stress.

Omega-3 Fatty Acids in Cardiovascular and Metabolic Disease Prevention, *chaired by* ***Maret Traber*** *(LPI) and* ***Pamela Starke-Reed*** *(NIDDK/National Institutes of Health)*

• **Richard Deckelbaum** (Columbia University) reviewed the health benefits of omega-3 fatty acids, commonly consumed in fish and fish oil supplements. Two important omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are inefficiently synthesized in the body from another dietary omega-3 fatty acid,

continued on page 2

Continued from cover — From the Director

Institute. In March, we met with key supporters of the Institute whom we asked the three questions with which I began this column. Next, we identified a steering committee consisting of select LPI faculty and staff, OSU's Vice President for Research and the former Dean of Research, and two donors and representatives from the private sector. Gathering the input of all LPI faculty was the next step, as well as the deans of the colleges comprising OSU's division of health sciences and the directors of related research centers and institutes on campus.

Taking the advice from all of these stakeholder groups under careful consideration, I prepared a document in which I identified the emerging key strategic areas for LPI's future and proposed eight task forces that each will develop three to five initiatives to address these key strategic areas in depth. The task forces are aligned with the major research and outreach/education programs in LPI, namely: Cancer Chemoprotection Program, Cardiovascular and Metabolic Diseases, Healthy Aging Program, Translational Research, Micronutrient Information Center, Healthy Youth Program, Communications, and LPI Operations. The task forces are chaired by LPI faculty and staff and consist of up to 10 members drawn from LPI faculty, staff, and students; other OSU faculty; and several outside experts, primarily scientific peers from other institutions.

As we keep up the momentum, I encourage you to contact me with questions and ideas about our strategic planning project. In the next Newsletter, I will update you on our progress and the emerging key initiatives for the Institute's future. In the meantime, I hope you enjoy reading this Newsletter, which contains summaries of our Diet and Optimum Health conference last May and recent publications by LPI investigators. As always, here's to your good health! LPI

Continued from cover — The Diet and Optimum Health Conference

alpha-linolenic acid found in flaxseed, walnuts, and vegetable oils. Omega-3 fatty acids play critical roles in cognitive and visual development, enhance immunity, delay neurodegeneration, and decrease inflammation. In mice, omega-3 fatty acids decrease cholesterol accumulation in arteries, protect against fatty liver, and, after acute administration, are neuroprotective after ischemic stroke. In humans, a high intake of omega-3 fatty acids is associated with a decreased risk for cardiac arrhythmia and sudden death from cardiovascular disease, and may help manage fatty liver disease.

• **William Harris** (Health Diagnostic Laboratory, VA) discussed non-fish sources of omega-3 fatty acids, such as krill, algae, and soybean oil containing stearidonic acid (SDA), which is more easily converted to EPA than the conversion of dietary alpha-linolenic acid to EPA. Consuming six grams of SDA-fortified soybean oil raised the EPA content of red blood cells as much as consuming one gram of pure EPA.

• **William Stanley** (University of Sydney, Australia) noted that supplementation with omega-3 fatty acids changes the fatty acid composition of cardiac cell membranes. This alteration decreases inflammation and is associated with protection against heart failure, although our understanding of the precise mechanisms is incomplete. Cardiac cells are rich in mitochondria, organelles that produce chemical energy in cells. DHA and EPA affect mitochondrial membrane function and improve resistance to apoptosis (programmed cell death) and, consequently, heart failure. A high intake of omega-3 fatty acids also decreases the risk for other cardiovascular events.

(left to right) Alan Taylor, Gary Merrill, and Donald Reed

• **Dariush Mozaffarian** (Harvard University) reported on the human studies investigating the role of omega-3 fatty acids in cardiovascular diseases. Results from such studies have been inconsistent but generally support a protective role for omega-3 fatty acids against cardiac arrhythmia and mortality. Studies indicate that there may be a threshold effect; intakes above this threshold are not associated with further improvements. He recommended to consume at least 250 mg/day of omega-3 fatty acids or at least two servings of oily fish per week.

• **Donald Jump** (LPI) addressed the role of omega-3 fatty acids in nonalcoholic fatty liver disease, which is associated with obesity. The progressive form of fatty liver disease (nonalcoholic steatohepatitis or NASH) can result in cirrhosis and liver cancer. In studies of mice fed a Western-type diet (high in saturated fat, sucrose, and cholesterol), supplementation with omega-3 fatty acids, especially DHA, attenuated inflammation, oxidative stress, and fibrosis and favorably affected patterns of gene expression and metabolism, suggesting that DHA may have value in preventing fatty liver disease.

Vitamin D—Health Benefits Beyond Bone,
*chaired by **Adrian Gombart** (LPI)*

• Modern lifestyles and geographical location influence the amount of vitamin D made in the skin. **Robert Heaney** (Creighton University) noted that vitamin D is involved in the expression of about 10% of our genes. Randomized controlled trials (RCTs) are not well suited to address quantitative issues concerning supplementation; assessments using physiological criteria may be better. Osteomalacia (bone pain) and inadequate calcium absorption may occur when serum levels of vitamin D are below 32 nanograms/milliliter, but studies suggest that higher levels may be needed for optimal insulin responsiveness and protection against certain cancers. Based on available evidence and estimated blood concentrations in our ancestors, 40-60 ng/mL may be optimal.

• **Thomas Burne** (The University of Queensland, Australia) discussed the importance of vitamin D in brain cells. Studies in Denmark found that infants with the lowest vitamin D blood levels had an increased risk for the later development of schizophrenia. In rats, vitamin D deficiency affects neurotransmitter activity, and deficiency during pregnancy adversely affects offspring. Other studies have associated low vitamin D levels with depression, brain dysfunction, and worse recovery from stroke.

• **David Feldman** (Stanford University) examined the role of vitamin D in cancer prevention, especially breast cancer. Epidemiological studies have found that vitamin D deficiency and obesity correlate with increased risk for breast cancer. In human breast cancer cells, vitamin D inhibits the expression of aromatase, an enzyme that stimulates estrogens in the breast, leading to cancer development. In obese and normal mice implanted with human breast cancer cells, vitamin D reduced tumor volume. In these experiments, vitamin D decreased aromatase and COX-2 enzymes, associated with inflammation. Vitamin D also has anti-proliferative functions, inhibits angiogenesis (blood vessel formation) required by tumors, and stimulates the differentiation, or normal maturation, of cells.

• Vitamin D deficiency is associated with an increased risk for cardiovascular diseases. **Stefan Pilz** (Medical University of Graz, Austria) noted that mortality from cardiovascular disease is highest in winter and in northern latitudes; both factors are associated with lower endogenous vitamin D synthesis. Obesity also causes vitamin D deficiency. The heart and vasculature have receptors for vitamin D; adequate vitamin D status lowers blood pressure and may protect against stroke and sudden cardiac death. Several large clinical trials of vitamin D supplementation and cardiovascular disease are ongoing, but the study populations were not selected for vitamin D deficiency, so results may not be definitive. Meta-analyses of other clinical trials have found that vitamin D supplementation decreases mortality, mainly caused by cardiovascular events.

continued on page 4

(left to right) David Ludwig, Edgar Miller III, Eric Rimm, and Fiona Harrison

Continued from page 3 — The Diet and Optimum Health Conference

Health Effects and Mechanisms of Action of Xanthohumol, *chaired by* ***Fred Stevens*** *(LPI)*

• When hyaluronan is overproduced by cartilage cells called chondrocytes, osteoarthritis may result. Inflammatory cytokines like IL-17 stimulate the production of hyaluronan in chondrocytes, a process that may also contribute to cancer metastasis and edema. In cell-culture experiments, **Peter Prehm** (Münster University Hospital, Germany) showed that the flavonoid xanthohumol, commonly extracted from hops used to make beer and from horny goat weed, inhibited hyaluronan export from cells and prevented collagen degradation. Curcumin from the spice turmeric also inhibited hyaluronan export from cells.

• Since the metabolite of an isomer of xanthohumol called isoxanthohumol is a phytoestrogen, **Richard van Breemen** (University of Illinois) has been studying its possible use in hormone-replacement therapy for menopausal symptoms. Xanthohumol and related compounds inhibited drug-metabolizing enzymes *in vitro*, suggesting the need for *in vivo* human studies. Xanthohumol is slowly absorbed into the blood stream and excreted in bile, with a half-life in the body of about 20 hours.

• **Fred Stevens** (LPI) noted that 2013 marks the 100th anniversary of xanthohumol research. Xanthohumol has anticancer, anti-inflammatory, and anti-hyperglycemic effects. In a rat model of metabolic syndrome, xanthohumol decreased body weight, fasting plasma glucose and fatty acid levels, and products of dysfunctional lipid metabolism. Xanthohumol also decreased reactive oxygen species by stimulating the synthesis of glutathione, an endogenous antioxidant.

Diet and Epigenetic Impacts on Disease and Aging, *chaired by* ***Rod Dashwood*** *(LPI)* and ***Joe Beckman*** *(LPI)*

• **Kent Thornburg** (Oregon Health & Science University) discussed the influence of fetal and maternal nutrition ("programming") and birth weight on the risk for chronic diseases later in life. Infants born to mothers who have either low- or high-caloric malnutrition have a higher risk for coronary heart disease and metabolic syndrome. Very low birth weights predict a higher risk for diabetes, hypertension, metabolic and neurological deficits, and cardiac mortality. Additionally, fetal nutrition affects subsequent generations—oocytes are formed in fetuses that later mature into eggs. Poor fetal nutrition can lead to structural abnormalities in organs, such as smaller hearts, or fewer neurons. For example, the number of cardiac muscle cells in adult sheep is predetermined during gestation, when proper nutrition is crucial.

Clarissa Gerhäuser and Jessica Keune

• **Sang-Woon Choi** (Tufts University) discussed the epigenetic impact of the chemical modification of DNA by methylation (addition of a CH_3 group) and hydroxymethylation (addition of a CH_2-OH group)—influenced by diet and age—that affects gene expression. Methylation involves several B vitamins and can be influenced by dietary factors like sulforaphane in broccoli or polyphenols in tea. Feeding alcohol to young and old mice reduced hydroxymethylation only in young mice, which may inhibit gene expression. Such epigenetic influences can determine the phenotype—how genes are expressed—resulting in alterations in vulnerability to disease.

• A dramatic example of epigenetic influence is the "Dutch Hunger Winter" of 1944-5 during which the Dutch faced famine, including micronutrient deficiencies, because of WWII. Sixty years later, the offspring of mothers who endured the famine had increased risk for obesity, cancer, and cardiovascular diseases. **Susan Duthie** (University of Aberdeen, UK) noted that folate deficiency is among the most common micronutrient deficiencies in the world and affects oxidative stress and gene expression through altered DNA methylation (an epigenetic event), synthesis, and repair. In mice fed a high-fat diet, folate deficiency accelerated atherosclerotic plaque development.

• **Donato Romagnolo** (University of Arizona) discussed the epigenetic modification of BRCA-1, a gene whose product —a tumor-suppressor protein—is decreased in sporadic breast cancer. A diet rich in fruits and vegetables decreases the risk of mutations in BRCA-1. The activity of the gene that triggers the synthesis of BRCA-1 may be increased by dietary factors, leading to the activation of tumor-suppressor genes and the silencing of oncogenes.

• **Clarissa Gerhäuser** (German Cancer Research Center, Heidelberg) studies the methylation of genes associated with breast and prostate cancer. Polyphenols in food and tea, selenium, curcumin, isothiocyanates and sulforaphane from cruciferous vegetables, and lycopene all affect DNA methylation *in vitro* and may reduce cancer risk. In cancer cells, hypermethylation of DNA may lead to increased metastasis. Gene methylation is often associated with changes in cell proliferation, growth, and death. Methylation inhibitors have been found to dose-dependently inhibit breast cancer *in vitro*.

• MicroRNAs are small non-coding RNA molecules that regulate gene expression. **Alberto Izzotti** (University of

Genoa, Italy) noted that microRNA activity can be modulated by vitamins A, B, D, and E; selenium; curcumin; resveratrol; catechins (polyphenols in tea, chocolate, grapes, berries); and indole-3-carbinol and isothiocyanates from cruciferous vegetables and discussed their importance in cancer chemoprevention. Some of these substances protect the enzyme DICER—involved in microRNA maturation—from being blocked by carcinogens like those in cigarette smoke or by mutagens in meat cooked at high temperatures.

Health Benefits of Vitamin C: Beyond Scurvy,

chaired by ***Balz Frei*** *(LPI)*

• Over 40 years ago, Linus Pauling and Ewan Cameron reported favorable responses in terminal cancer patients receiving vitamin C. **Garry Buettner** (University of Iowa) reviewed our current understanding of the anticancer mechanisms of vitamin C. Despite the powerful antioxidant properties of vitamin C, in very high concentrations it can produce hydrogen peroxide in the body that can kill susceptible cancer cells. High oral doses of vitamin C can raise plasma levels to about 80 micromolar, but intravenous infusions of 100 grams raise plasma concentrations to more than 250 times greater

(left to right) Carmen Wong, Lauren Atwell, Emily Ho, Laura Beaver, and Gregory Watson

(20 millimolar). In a small clinical trial in patients with pancreatic cancer, large infusions (up to 100 grams) of vitamin C combined with gemcitabine have resulted in increased survival times. It's uncertain if vitamin C is selectively cytotoxic to cancer cells or mainly cytostatic. Vitamin C infusions also decrease levels of F_2-isoprostanes, biomarkers of oxidative stress.

• **Edgar Miller, III** (Johns Hopkins University) conducted a meta-analysis of 29 clinical trials (with a total of over 1,400 subjects) on vitamin C and blood pressure reported between 1966 and 2011. The median dose of vitamin C was 500 mg/day for a median duration of eight weeks. Vitamin C supplements reduced systolic blood pressure (SBP) by 3.8 mm Hg and diastolic blood pressure (DBP) by 1.5 mm Hg. In hypertensive subjects, vitamin C reduced SBP by 4.9 mm Hg and DBP by 1.7 mm Hg. In other short-term studies, vitamin C has lowered uric acid levels and improved flow-mediated vasodilation, or arterial relaxation. Long-term trials are needed to determine the duration of benefit.

(left to right) Adrian Gombart, Maret Traber, and Barbara McVicar

• **Fiona Harrison** (Vanderbilt University) addressed the role of vitamin C in brain aging and cognitive function. Vitamin C is an antioxidant and participates in neurotransmitter synthesis. In mouse models of aging and Alzheimer's disease, parenteral administration of vitamin C by injection or intravenous infusion improves learning and memory. In the Alzheimer's disease model, a 25% decrease in vitamin C levels in the brain is associated with oxidative damage and beta-amyloid pathology. In a test of physical stamina, mice with low vitamin C levels performed worse than those with higher levels. Based on NHANES 2009-10 data, as much as 30% of the American population may be vitamin C inadequate.

Micronutrients in Fertility and Pregnancy,

chaired by ***David Williams*** *(LPI)*

• **Helene McNulty** (University of Ulster, Northern Ireland) discussed the critical role of maternal folate intake in preventing neural tube defects and, possibly, facial clefts and heart defects in infants. The neural tube in embryos closes at about the time when a woman may suspect that she is pregnant, so consistent supplementation through fortified foods or a multivitamin/mineral supplement during periconception is important. In clinical trials, supplemental folic acid reduced stroke incidence by about 20% in people without a history of stroke. The B vitamin riboflavin may lower blood pressure, depending on genotype. Folate status is inversely correlated with homocysteine levels, which are associated with an increased risk for cardiovascular diseases. Fortified food contains folic acid, which is more bioavailable than the folate found naturally in food. Food folate can be destroyed by cooking, and food sources are limited. Folic acid converts to folate in the body in about 90 minutes.

continued on page 6

Continued from page 5 — The Diet and Optimum Health Conference

(left to right) Ralph Reed, Bob Durst, and Biff Traber

• **Kelton Tremellen** (University of South Australia) talked about the role that micronutrients play in fertility and early fetal development. Oxidative stress adversely affects the quality and motility of sperm, as well as DNA integrity. Antioxidants like vitamins C and E, zinc, selenium, carotenoids, and lycopene can attenuate oxidative damage and improve fertility. Oxidative stress is also implicated in erectile dysfunction and female reproductive abnormalities. Additionally, low levels of vitamin D in pregnancy are associated with an increased risk for asthma and schizophrenia in offspring. Low maternal levels of folate, which can affect methylation, are linked to a higher risk for miscarriage. Low-iodine levels are associated with decreased sperm quality. While excess iron may cause adverse effects, appropriate iron levels help in ovulation.

• **Victoria Moran** (University of Central Lancashire, UK) addressed the importance of micronutrients like iron, iodine, and zinc on brain development in the fetus. Over 50% of premenopausal women have insufficient iron status, and iron-deficient anemia is prevalent around the world. Iron is important in energy metabolism, neurotransmitter function, and myelin synthesis. While animal studies indicate that maternal iron deficiency results in irreversible deficits in offspring, human studies show that iron treatment in iron-deficient children improves cognitive development and function. Although there is no good biomarker for zinc status in humans, zinc deficiency in animals results in poor attention and learning. There are "windows of sensitivity" during which micronutrient status importantly affects fetal development. The magnitude of the effect of micronutrient status in infants and children may be influenced by context—poverty, nutrient interactions, multiple deficiencies, parent-infant relationships, physical closeness between parents and infants, and breast-feeding, which can be a good source of the omega-3 fatty acid docosahexaenoic acid important in brain development.

Oral Abstracts, *chaired by* ***Viviana Perez*** *(LPI) and* ***Kathy Magnusson*** *(LPI)*

Eight posters were selected for oral presentation:

• For the Oregon Brain Aging Study, **Gene Bowman** (Oregon Health & Science University) measured omega-3 fatty acids (eicosapentaenoic acid and docosahexaenoic acid) in the plasma of older adults without dementia or Alzheimer's disease. Those subjects with the highest levels of omega-3 fatty acids had a delay of one year in the decline of age-related, executive cognitive function, possibly due to a reduction in the number of brain lesions.

• Previous research has found that a multivitamin/mineral supplement (MVM) containing guarana, a fruit from trees native to the Amazon, improved executive cognitive and memory functions. The present study by **Andrew Scholey** (Swinburne University, Australia) addressed whether guarana or the MVM was responsible for those effects in 20 healthy, young adults. Subjects who got the MVM with guarana showed improved attention, memory performance, and mood compared to those who got a placebo. Compared to placebo, the MVM with and without guarana increased brain activity in the area associated with memory, which was more pronounced in those taking the MVM with guarana.

Elisa Monaco and child

• In a study reported by **Özgür Sancak** (Bayer Consumer Care, Switzerland), the effect of vitamin C on gene expression and inflammation was examined in five healthy subjects who got one gram daily of vitamin C for five days. Blood taken from the subjects and exposed to an inflammatory stimulus exhibited a pattern of gene expression different from that in unstimulated blood; specifically, vitamin C increased the amount of interleukin 10, an anti-inflammatory cytokine, and decreased NF-κB, a marker of inflammation.

(left to right) Kimberly Holmes, Candace Russo, Debbie Mustacich, Kate Shay, and Dove Keith

• Cancer cells in a solid tumor become hypoxic (oxygen deprived) because of inadequate angiogenesis (blood vessel formation), which affects their response to chemotherapy and radiation. Hypoxia-inducible factor-1 (HIF-1), which is elevated in hypoxic tumor cells, mediates angiogenesis and glucose transport and can be turned off by reactions that require vitamin C. After analyzing samples of normal and colorectal cancer tissue from 50 patients, **Margreet Vissers** (University of Otago, New Zealand) reported that vitamin C levels were lower in tumor tissue and correlated inversely with HIF-1 activity, tumor size, and necrosis. High levels of vitamin C in tumor cells were also associated with longer patient survival.

• **Laura Beaver** (LPI) discussed the effect of sulforaphane from cruciferous vegetables on gene expression in normal and cancerous prostate cells. Sulforaphane altered expression of about 3,000 genes, especially those involved in cell cycle, proliferation, apoptosis, and angiogenesis in cancer cells, thereby killing cancer cells but not normal cells. Its effects vary depending on the specific stage of carcinogenesis.

• Polyphenols in fruits and vegetables and their metabolites are associated with some protection against colon cancer. **Joanna Kaniewska** (University of Aberdeen, UK) examined the effect of polyphenols in a diet enriched with 7.5 servings of fruits and vegetables per day on gene regulation in normal human colon cells, urine, plasma, and feces collected from 21 human subjects who consumed the enriched diet for 12 weeks. Various polyphenols were associated with increased antioxidant protection and DNA stability.

• High consumption of soy is associated with a lower incidence of breast cancer in Asian women. **Ben O. de Lumen** (University of California-Berkeley) discussed the role of lunasin, a peptide found in soy, peanuts, and seeds and in the Soy Bowman Birk Inhibitor (BBI) Concentrate currently being tested in clinical trials, on human breast cancer cells implanted into mice. Intraperitoneally injected lunasin, but not BBI, effectively inhibited tumor growth and killed cancer cells. However, BBI protects lunasin from digestion when eaten, thereby improving its bioavailability.

• Levels of klotho, a protein found in cell membranes, decline with age. Klotho-deficient mice exhibit short lifespan, kidney disease, osteoporosis, skin atrophy, and dysregulation of vitamin D. **John Finnell** (AOMA Graduate School of Integrative Medicine, Texas) gave 10,000 IU/day of vitamin D_3 for 12 weeks to 40 adults with vitamin D insufficiency (<30 ng/mL) and then measured plasma klotho concentrations. Plasma levels of vitamin D increased after supplementation, and levels of klotho significantly increased as well.

continued on page 8

(left to right) Louis Barbeito, Joe Beckman, and Gary Merrill

Continued from page 7 — The Diet and Optimum Health Conference

The Young Investigator Awards were presented to (left to right) Molly Derry, Rachel Botchlett, and Erica Sharpe.

Young Investigator Awards

Four young scientists were selected for Young Investigator Awards:

• **Molly Derry** (University of Colorado), for her presentation "Investigating grape seed extracts chemopreventive efficacy in *in-vitro* and *in-vivo* models of colorectal cancer"

• **Erica Sharpe** (Clarkson University), for "Effect of brewing conditions and re-infusion on the antioxidant capacity of green tea: A side-by-side analysis using the ORAC and NanoCerac assays"

• **Rachel Botchlett** (Texas A&M University), for "Effects of a high-fat diet and metformin treatment on sarcolemmal and insulin signaling protein expression in young mice"

• **Yasmeen Nkrumah-Elie** (LPI), for "Biomarkers of zinc deficiency"

Public Session

Whole-food Approaches to Disease Prevention,
chaired by ***Emily Ho*** *(LPI) and* ***Balz Frei*** *(LPI)*

• **Penny Kris-Etherton** (The Pennsylvania State University) reviewed the influence of whole grains and nuts on cardiovascular and metabolic diseases. Ischemic heart disease and stroke have been identified as, respectively, the number one and number three health problems in the world. Significant risk factors include high blood pressure and low consumption of whole grains, seeds, and nuts. Many studies have shown that high consumption of whole grains, including wheat, oats, rye, rice, and popcorn, is associated with lower body mass index (BMI), blood pressure, and cholesterol levels, and a decreased risk for metabolic syndrome. One study with 50 adults given six servings of refined or whole grains per day for 18 weeks found that whole grains reduced the prevalence of pre-diabetes by 90%. Nut consumption reduces the risk for cardiovascular disease and associated morbidity and mortality in a dose-response manner. Nuts also reduce cholesterol and triglycerides, lower blood pressure, reduce oxidative stress and inflammation, and improve vascular function.

• **Eric Rimm** (Harvard University) discussed the health effects of polyphenols present in fruits, vegetables, and some beverages. Different polyphenols have subtle differences in chemical structure that affect their biological activity and health benefits. Phenolic acids are found in olives, coffee, wine, nuts, and spices. Berries, wine, and nuts contain stilbenes. Lignans are present in sesame and olive oils, flaxseed, and whole grains. Flavonoids are found in berries, apples, chocolate, wine, and tea. Generally, polyphenols improve vascular function, lower blood pressure and lipids, and help with glycemic control. Processing, including cooking, and exposure to sunlight affect the concentration of polyphenols in food. Blueberries and strawberries contain a flavonoid called anthocyanin, and in a large study, blueberries (2-4 servings per week) were found to be especially effective in lowering the risk for hypertension, diabetes, stroke, and heart disease.

• **Cynthia Thomson** (University of Arizona) emphasized that dietary habits can affect the risk for breast cancer and metabolic syndrome that is associated with diabetes risk. Breast cancer is the most common type of cancer in women, and among 35- to 55-year-old American women, it is the leading cause of death. Several themes to reduce breast cancer risk emerged from her presentation: limit alcohol and eat nutrient-dense food, less fat, more vegetables high in carotenoids, spices, and high-fiber food. Metabolic syndrome is characterized by high blood sugar, elevated lipids, large waist circumference, and high blood pressure. If not corrected, it can progress to diabetes. Avoiding simple sugars and consuming high-fiber foods are protective. Maintaining a good BMI helps to prevent breast cancer and metabolic syndrome.

• The incidence of childhood obesity is rising in the US, which may shorten life expectancy by two to five years. It affects not only all the body's organs but also psycho-social development. **David Ludwig** (Harvard University) discussed the importance of an integrated, family-based approach to solving this problem. Children may express preference for sweet, salty, and fatty foods, but this is largely programmed by adults. Establishing a parenting style that favors health is the key to successful weight management in children. A diet low in simple carbo-hydrates is better than a low-fat diet for losing weight, since calories from fat have declined while obesity rates have increased. Exercise in children may not overcome the effect of overwhelming caloric intake, but, if regular and fun, it may help. LPI

The Linus Pauling Institute Prize for Health Research

Helmut Sies (left) receives the LPI Prize from Balz Frei.

At the Diet and Optimum Health Conference, the seventh Linus Pauling Institute Prize for Health Research was presented to Dr. Helmut Sies of Heinrich Heine University Düsseldorf in Germany. The Prize, consisting of a medal and $25,000, 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. The prize recognized Dr. Sies' important discoveries relating to the antioxidant and skin protective effect of the carotenoid lycopene, the role of flavanols in cocoa on vascular function, and the biochemistry and antioxidant function of selenium-containing proteins. In 1985, Dr. Sies coined the term "oxidative stress," which refers to an imbalance between antioxidants and reactive oxygen species/oxidants that can damage biomolecules like DNA, proteins, fatty acids, and cell membranes. As one prominent researcher noted in his letter of support for Dr. Sies' nomination, "Dr. Sies has contributed so heavily to the phytochemical/nutrient health promotion and oxidative stress/antioxidant areas that he is considered as both a pioneer and a giant in all these fields."

Dr. Sies received his medical degree from the University of Münich and held faculty positions at the University of Münich before being appointed Professor and Chairman in the Department of Physiological Chemistry at the University of Düsseldorf. He has also served as an adjunct Professor in the Department of Molecular Pharmacology and Toxicology at the University of Southern California since 2000. He is the author of over 500 scientific papers and over 25 books.

In his lecture after accepting the award, Dr. Sies discussed his research interests in depth, including the topic of oxidative stress. Reactive oxygen species (ROS)—molecules with unpaired electrons that "steal" electrons from other molecules, thereby oxidizing them—can be deleterious or beneficial, depending on the context. For example, tadpole tail regeneration after amputation requires ROS. Plants synthesize antioxidants like vitamin C and carotenoids to protect against oxidative damage from the sun's ultraviolet light.

Dr. Sies mentioned that Albert Szent-Györgyi, who won the 1937 Nobel Prize in Physiology or Medicine for work on vitamin C and oxidation reactions, originally proposed the term "ignose" for vitamin C, underscoring some ignorance about the molecule, then "godnose," followed by a compromise with "hexuronic acid," which turned out to be incorrect, and finally, with Haworth, "ascorbic acid."

Singlet oxygen, an ROS produced in plants by the action of chlorophyll and also present in the human body, is most effectively quenched by the carotenoid lycopene, which Dr. Sies has studied for many years. He carried out seminal research on the absorption of lycopene, its biochemistry, and its health effects, particularly its protection against UV-induced erythema or sunburn.

Arteries expand and contract depending on many factors, and good arterial dilation is associated with normal blood flow. Arterial dilation is impaired after consuming a high-fat meal that causes oxidative stress and the generation of oxidized fats, which can contribute to the atherosclerotic process. Dr. Sies noted that consumption of red wine attenuates the increase in lipid oxidation and that arterial dilation after such a high-fat meal can be improved by polyphenols, especially, as Dr. Sies' research has demonstrated, flavanols in chocolate. Some of these flavanols, such as the catechins, are also found in tea. Cocoa can also attenuate UV-induced erythema. Dr. Sies referred to these beneficial effects of polyphenols as "metabolic fine tuning."

Selenium plays an important role in the function of the endogenous antioxidant glutathione. Dr. Sies has published much research on glutathione peroxidase, a selenium-containing enzyme that protects against oxidative damage. Glutathione peroxidase helps to get rid of hydrogen peroxide, which itself is not a free radical but can generate damaging free radicals when it reacts with some forms of metals like copper and iron to form hydroxyl radicals. In related work, Dr. Sies detected the "nitration" of proteins ("nitrative stress") by nitrogen radicals implicated in cellular dysfunction and disease, including amyotrophic lateral sclerosis (ALS). Nutritionally adequate selenium may be helpful in the prevention of diabetes, but, as Dr. Sies has pointed out, excessive levels may actually increase the risk for diabetes. LPI

Selected Recent Publications by LPI Scientists

Summarized by Stephen Lawson, LPI Administrative Officer
LPI scientists in boldface

Healthy Aging Program

GUO C, ROSOHA E, **LOWRY MB**, BORREGAARD N, and **GOMBART AF**. Curcumin induces human cathelicidin antimicrobial peptide gene expression through a vitamin D receptor-independent pathway. *J. Nutr. Biochem*. **24**:754-759, 2013

Vitamin D induces the synthesis of an antimicrobial peptide in the body called cathelicidin, which attacks pathogenic microbes. In cell-culture studies, the authors found that polyunsaturated fatty acids did not induce cathelicidin but that curcumin—derived from the spice turmeric—did, although not as strongly as vitamin D. This induction was independent of the vitamin D receptor, and its mechanism is currently being investigated in the Gombart lab.

GUO C, **SINNOTT B**, NIU B, **LOWRY MB**, **FANTACONE ML**, and **GOMBART AF**. Synergistic induction of human cathelicidin antimicrobial peptide gene expression by vitamin D and stilbenoids. *Mol. Nutr. Food Res*. 1-9, 2013

Using human cells in culture, the authors screened 446 molecules being investigated in human clinical trials to find ones that affect the regulation of the cathelicidin antimicrobial peptide gene. Two candidates induced cathelicidin: the stilbenoid resveratrol in red grapes and wine and pterostilbene, found in blueberries. Each compound also acted synergistically with vitamin D to further increase cathelicidin levels. More research is necessary to determine if these effects can be achieved in humans.

CAMPBELL Y, **FANTACONE ML**, and **GOMBART AF**. Regulation of antimicrobial peptide gene expression by nutrients and by-products of microbial metabolism. *Eur J. Nutr*. **51**:899-907, 2012

In this paper, the authors review the effect of nutrients and by-products of gut microbes on the regulation of antimicrobial peptides like defensin and cathelicidin synthesized in immune and epithelial cells. Vitamins A and D upregulate genes for the production of anti-microbial peptides important in innate immunity. Additionally, butyrate, a fatty acid formed in the gut by the bacterial metabolism of fiber, effectively increases antimicrobial peptide synthesis, especially cathelicidin. In rabbits, butyrate-induced stimulation of cathelicidin has been shown to ameliorate intestinal shigella infection. Bile acids also increase cathelicidin, possibly explaining the sterility of the bile duct. Sulforaphane, found in cruciferous vegetables like broccoli, has been shown to induce defensins in cultured human colon cells.

DIXON BM, BARKER T, MCKINNON T, CUOMO J, **FREI B**, BORREGAARD N, and **GOMBART AF**. Positive correlation between circulating cathelicidin antimicrobial peptide (hCAP18/LL-37) and 25-hydroxyvitamin D levels in healthy adults. *BMC Res. Notes* **5**:575-579, 2012

Vitamin D strongly induces the antimicrobial peptide cathelicidin. The authors measured blood levels of vitamin D and cathelicidin in 19 healthy, middle-aged men and women. Serum levels of vitamin D up to 32 ng/mL were positively correlated with increasing concentrations of cathelicidin, although there was no correlation at serum levels of vitamin D above 32 ng/mL. This study suggests that protection against infection or sepsis may be increased by vitamin D supplementation or sun exposure that raises serum levels of vitamin D to 32 ng/mL.

FOK WC, ZHANG Y, SALMON AB, BHATTACHARYA A, GUNDA R, JONES D, WARD W, FISHER K, RICHARDSON A, and **PEREZ VI**. Short-term treatment with rapamycin and dietary restriction have overlapping and distinctive effects in young mice. *J. Gerontol. A. Biol. Sci. Med. Sci*. **68**:108-116, 2013

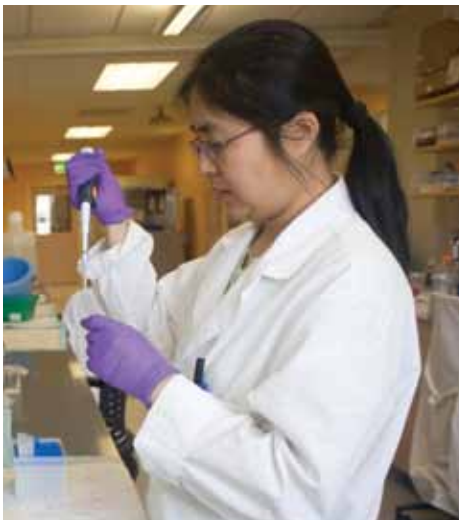
Rapamycin is a natural antifungal, antibiotic substance found in the soil on Easter Island. Rapamycin and caloric restriction both increase the lifespan of mice by inhibiting cell growth and increasing autophagy (the degradation of dysfunctional cells). Unlike rapamycin, caloric restriction improved insulin sensitivity and glucose handling, attenuated weight gain, improved antioxidant mechanisms, and decreased adiposity in mice.

FRANCO MC, YE Y, REFAKIS CA, FELDMAN JL, STOKES AL, BASSO M, MELERO FERNANDEZ DE MERA RM, SPARROW NA, CALINGASAN NY, KIAEI M, **RHOADS TW**, MA TC, GRUMET M, BARNES S, BEAL MF, **BECKMAN JS**, MEHL R, and ESTEVEZ AG. Nitration of Hsp90 induces cell death. *Proc. Natl. Acad. Sci. USA* **110**:1102-1111, 2013

Peroxynitrite is a potent oxidant formed from the reaction between superoxide and nitric oxide that can damage proteins, leading to the death of motor neurons, through a process called nitration. The heat shock protein Hsp90 is synthesized in cells to help fold and stabilize more than 200 proteins involved in cell survival and death. In this study, the authors showed that nitration of a single amino acid (tyrosine) in Hsp90 changes it from a pro-survival protein to a mediator of motor neuron death. Nitrated Hsp90 was found in ALS patients and in cases of spinal cord injury, further implicating peroxynitrite in neuronal cell death and damage.

KEITH DJ, **BUTLER JA**, **BEMER B**, DIXON B, JOHNSON S, **GARRARD M**, SUDAKIN DL, CHRISTENSEN JM, PEREIRA C, and **HAGEN TM**. Age and gender dependent bioavailability of *R*- and *R,S*-α-lipoic acid: A pilot study. *Pharmacol. Res*. **66**:199-206, 2012

R-alpha lipoic acid is a naturally occurring substance synthesized in the body and present in food like spinach and broccoli. Lipoic acid is an antioxidant and plays a role in the cell's energy metabolism in mitochondria. In old rats, lipoic acid supplementation increases other antioxidant levels and protects against age-related stress. It detoxifies heavy metals, ameliorates inflammation, and has been used in people to treat diabetic neuropathy. Commercially made lipoic acid is a racemic mixture of the *R* and *S* forms. In this study, the authors gave 500 mg of either *R*-alpha-lipoic acid or *R,S*-alpha-lipoic acid to 10 young adults (18-45 years old) and nine elderly adults (over 75 years old) to determine how much of the different forms was absorbed into the blood stream. Absorption was quite variable. Generally, bioavailability of both forms was about the same in older and younger adults.



KYME P, THOENNISSEN NH, TSENG CW, THOENNISSEN GB, WOLF AJ, SHIMADA K, KRUG UO, LEE K, MULLER-TIDOW C, BERDEL WE, HARDY WD, **GOMBART AF**, KOEFFLER HP, and LIU GY. C/EBPε mediates nicotinamide-enhanced clearance of *Staphylococcus aureus* in mice. *J. Clin. Invest*. **122**:3316-3329, 2012

The authors found that vitamin B_3 (niacin), when injected into mice infected with the bacterium *Staphylococcus aureus*, enhanced the killing of *S. aureus* up to 1,000-fold. Pretreatment of human blood with B_3 greatly reduced the survival of added *S. aureus*. B_3 had no effect in mice without neutrophils, suggesting that these immune cells are crucial for the antibacterial effect. In contrast to mice, B_3 stimulated the production of an antimicrobial peptide in human neutrophils. These results suggest that safely achievable levels of B_3 may have therapeutic value in treating certain infections.

WONG CP, **MAGNUSSON KR**, and **HO E**. Increased inflammatory response in aged mice is associated with age-related zinc deficiency and zinc transporter dysregulation. *J. Nutr. Biochem*. **24**:353-359, 2013

Aging is associated with a decline in the performance of the immune system. The authors used cell cultures and mice to study the effect of zinc on immunity and found that age-related zinc deficiency in immune cells and dysregulation of zinc transporters impaired immune function and increased inflammation. Epigenetic influences, especially the under- or over-methylation of DNA, also contributed to the age-related decline in zinc status and enhanced inflammation. Zinc supplementation of old mice consuming a zinc-adequate diet dramatically reduced serum levels of inflammatory markers like IL-6 and TNF-α.

Cardiovascular and Metabolic Diseases

FREI B, BIRLOUEZ-ARAGON I, and LYKKESFELDT J. Authors' perspective: What is the optimum intake of vitamin C in humans? *Crit. Rev. Food Sci. Nutr*. **52**:815-829, 2012

Linus Pauling's favorite molecule—vitamin C—is required to prevent scurvy but has other important effects: enhancing the absorption of nonheme iron, scavenging free radicals as the premier antioxidant, helping to synthesize neurotransmitters and carnitine, and detoxifying histamine. The authors reviewed the evidence for vitamin C's role in the primary prevention of heart disease, stroke, and cancer, concluding that 200 mg/day may be optimum. Vitamin C reduces hypertension, improves endothelial function or arterial relaxation, and decreases chronic inflammation, a hallmark of chronic diseases. They note that standard clinical trials designed to assess drug efficacy aren't appropriate to study micronutrients like vitamin C for many reasons, including the lack of a true placebo group. Instead, they argue that the optimum intake of vitamin C can be deduced from all the available evidence from clinical trials and observational studies, as well as based on biological plausibility.

DEPNER CM, PHILBRICK KA, and **JUMP DB**. Docosahexaenoic acid attenuates hepatic inflammation, oxidative stress, and fibrosis without decreasing hepatosteatosis in a *Ldlr(-/-)* mouse model of Western diet-induced nonalcoholic steatohepatitis. *J. Nutr.* **143**:315-323, 2013

Nonalcoholic steatohepatitis (NASH), marked by liver damage, inflammation, oxidative stress, and fibrosis, is associated with obesity. Using a mouse model of NASH induced by feeding a Western-type diet high in fat and carbohydrates, the authors showed that supplementation with physiologically relevant doses of docosahexaenoic acid (DHA), an omega-3 fatty acid found in fatty fish, attenuated all of the markers of NASH. DHA was more effective than eicosapentaenoic acid (EPA), which is a precursor for DHA synthesis in the body.

WEI H, **ZHANG W**, MCMILLEN TS, LEBOEUF RC, and **FREI B**. Copper chelation by tetrathiomolybdate inhibits vascular inflammation and atherosclerotic lesion development in apolipoprotein E-deficient mice. *Atherosclerosis* **223**:306-313, 2012

Copper is an essential nutrient, like iron, that has been implicated in the inflammatory process of atherosclerosis. Adhesion molecules synthesized by surface cells of blood vessels and arteries can cause white blood cells called monocytes to adhere to these surface cells. The monocytes

continued on page 12

Continued from page 11 — Selected Recent Publications

then migrate into the intima and become macrophages that engulf oxidized cholesterol, leading to the formation of "foam" cells, a hallmark of atherosclerotic plaque formation. Tetrathiomolybdate (TTM) is a small molecule containing sulfur and molybdenum that has a strong binding affinity for copper. The authors showed that treating mice that develop atherosclerosis with TTM dramatically reduced copper levels in the aorta and heart, and, consequently, inhibited atherosclerosis. TTM did not affect iron homeostasis or reduce oxidative stress; rather, it chelated and removed copper, thereby limiting vascular inflammation.

MILLER GW, ULATOWSKI L, **LABUT EM**, **LEBOLD KM**, MANOR D, ATKINSON J, BARTON CL, TANGUAY RL, and **TRABER MG**. The α-tocopherol transfer protein is essential for vertebrate embryogenesis. *PLoS ONE*: **7**:e47402, 2012

The alpha-tocopherol transfer protein (TTP) recognizes only the alpha-tocopherol form of vitamin E for distribution to tissues. TTP is present in zebrafish, which are a good model to study embryogenesis and fetal development. Inhibition of TTP in zebrafish embryos results in severe malformations of the head and eyes, suggesting that TTP plays a crucial role in delivering vitamin E necessary for the proper development of the vertebrate central nervous system.

MICHELS AJ, **HAGEN TM**, and **FREI B**. Human genetic variation influences vitamin C homeostasis by altering vitamin C transport and antioxidant enzyme function. *Ann. Rev. Nutr*. **33**:45-70, 2013

The absorption of vitamin C from the gut into the blood stream is regulated by vitamin C transport molecules on the cells that line the intestine. Other factors, such as oxidative stress, inflammation, and enzyme activity that uses up vitamin C, also influence vitamin C levels in blood and tissues. This paper reviews the subtle differences in genes that code for the vitamin C transporters. These differences, called single-nucleotide polymorphisms (SNPs), result in different vitamin C dynamics among people. When possible, these SNPs should be taken into consideration when selecting populations to study the effect of vitamin C on chronic disease risk.

LEBOLD KM, LÖHR CV, BARTON CL, **MILLER GW**, **LABUT EM**, TANGUAY RL, and **TRABER MG**. Chronic vitamin E deficiency promotes vitamin C deficiency in zebrafish leading to degenerative myopathy and impaired swimming behavior. *Comp. Biochem. Physiol*. **157**:382-389, 2013

As in humans, ascorbic acid and alpha-tocopherol are vitamins for zebrafish. The authors fed diets with or without vitamin E to zebrafish for a year, with decreasing amounts of vitamin C in the diets for the last 150 days. At the end of the experiment, swimming behavior in response to a stimulus was measured. The zebrafish fed the diet without vitamin E exhibited high levels of oxidative stress markers and sluggish swimming behavior associated with myopathy of skeletal muscles. They also had lower levels of vitamin C than the fish fed vitamin E. The low levels of dietary vitamin C were insufficient to recycle vitamin E from its oxidized to reduced state.

TRABER MG. Mechanisms for the prevention of vitamin E excess. *J. Lipid Res*. **54**:2295-2306, 2013

Alpha-tocopherol is the only one of the eight forms of vitamin E that is recognized by the Institute of Medicine to meet vitamin E requirements in humans. Vitamin E is a fat-soluble antioxidant that protects polyunsaturated fatty acids from oxidation. Despite being fat-soluble, the alpha-tocopherol form of vitamin E does not accumulate in the liver to harmful levels. Alpha-tocopherol is selected by the alpha-tocopherol transfer protein—synthesized in the liver—for distribution to lipoproteins circulating in blood. Other forms of vitamin E are rapidly metabolized by cytochrome P450 enzymes in the liver, and the metabolites are excreted. Alpha-tocopherol does not accumulate in the liver because excessive amounts are either metabolized or excreted in bile.

JUMP DB, **DEPNER CM**, and **TRIPATHY S**. Omega-3 fatty acid supplementation and cardiovascular disease. *J. Lipid Res*. **53**:2525-2545, 2012

Consumption of fatty fish containing omega-3 fatty acids has been recommended for many years to help prevent cardiovascular diseases. In this paper, the authors review the evidence for the effect of omega-3 fatty acids like docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) on the primary and secondary (therapeutic) protection against cardiovascular diseases, as well as discuss the mechanisms that account for these effects. Omega-3 fatty acids reduce blood triglyceride levels, attenuate inflammation, and are anti-arrhythmic. DHA changes the fluidity of cell membranes and their cholesterol content. By improving mitochondrial function in cardiac cells, DHA has been found to help protect against cardiac arrhythmia, which is the main cause of sudden cardiac death. Omega-3 fatty acids have generally been shown to help prevent cardiovascular disease, whereas randomized clinical trials to test the effect on clinical events in patients with cardiovascular disease (secondary prevention) have had inconsistent results. DHA is poorly synthesized in the body from alpha-linolenic acid (ALA), the major source of omega-3 fatty acids in plants, algae, and yeast. ALA consumption does little to protect against cardiovascular disease.

Cancer Chemoprotection Program

PARASRAMKA MA, DASHWOOD WM, WANG, R, ABDELLI A, BAILEY GS, WILLIAMS DE, HO E, and **DASHWOOD RH.** MicroRNA profiling of carcinogen-induced rat colon tumors and the influence of dietary spinach. *Mol. Nutr. Food Res*. **56**:1259-1269, 2012.

MicroRNAs are small pieces of RNA that affect translation, the process by which messenger RNA codes for proteins after being transcribed from DNA. Dysregulation of microRNAs is associated with cancer and other pathologies. In this year-long study, the authors investigated the role of microRNA dysregulation in the development of colon cancer in rats induced by exposure to heterocyclic amines (cooked-meat mutagens) found in proteinacious food cooked at high temperatures. After the exposure to heterocyclic amines for several weeks (post-initiation), rats that were fed a diet containing freeze-dried spinach for the remainder of the experiment had a lower incidence of colon tumors, which correlated with the partial reversal of microRNA dysregulation.

CLARKE JD, RIEDL K, BELLA D, SCHWARTZ SJ, **STEVENS JF**, and **HO E.** Comparison of isothiocyanate metabolite levels and histone deacetylase activity in human subjects consuming broccoli sprouts or broccoli supplement. *J. Agric. Food Chem*. **59**:10955-10963, 2011

DNA in cells is wrapped around proteins called histones. When chemical acetyl groups are added to histones (acetylation), genes are turned on; when acetyl groups are removed (deacetylation), genes are turned off. Inhibitors of deacetylation cause tumor-suppressor genes to remain active. Glucosinolates are phytochemicals found only in cruciferous vegetables like broccoli. When such vegetables are chopped or chewed, an enzyme called myrosinase is released that converts the glucosinolates into a number of compounds, including an isothiocyanate called sulforaphane, which inhibits histone deacetylation. Gut bacteria also synthesize myrosinase. In this paper, the authors measured the amount of glucosinolate metabolites like sulforaphane in the urine of subjects consuming broccoli sprouts or a broccoli supplement and also assessed histone deacetylase activity in blood cells. Consuming broccoli sprouts was much more effective in elevating sulforaphane levels in urine and in decreasing histone deacetylase activity in blood cells, probably because of the absence of myrosinase in the broccoli supplement.

CLARKE JD, HSU A, YU Z, DASHWOOD RH, and **HO E.** Differential effects of sulforaphane on histone deacetylases, cell cycle arrest and apoptosis in normal prostate cells versus hyperplastic and cancerous prostate cells. *Mol. Nutr. Food Res*. **55**:999-1009, 2011

Sulforaphane is an isothiocyanate present in cruciferous vegetables that has previously been identified as an anticancer phytochemical due to its influence on phase 2 enzymes that modify xenobiotics and toxins for excretion from the body. More recently, sulforaphane has been found to act as a histone deacetylase inhibitor. In this role, sulforaphane helps to preserve the acetylation of

histones surrounding DNA, leading to the activation of tumor-suppressor genes. The authors exposed normal, benign hyperplastic, and cancerous prostate cells *in vitro* to sulforaphane, which acted as a histone deacetylase inhibitor and induced cell-cycle arrest and apoptosis (programmed cell death) in the hyperplastic and cancerous cells but not in the normal cells.

RAJENDRAN P, HO E, WILLIAMS DE, and **DASHWOOD RH.** Dietary phytochemicals, HDAC inhibition, and DNA damage/repair defects in cancer cells. *Clin. Epigenetics* **3**:4, 2011

In this review, the authors discuss the role of phyto-chemicals in cancer prevention, focusing on several mechanisms by which these phytochemicals lead to the impairment or death of cancer cells. Tea polyphenols, curcumin in the spice turmeric, indole-3-carbinol and isothiocyanates like sulforaphane in cruciferous vegetables, selenium, allium in garlic, genistein in soy, and quercetin in apples and onions can affect gene expression by altering the activity of histones, which are molecules around which DNA is wound. By inhibiting a chemical modification called histone deacetylation, these phytochemicals can turn on tumor-suppressor genes and affect DNA damage status. They can cause breaks in DNA through the generation of reactive oxygen species—easily repaired in normal cells but not in cancer cells. Most can aid in DNA repair in normal cells, and many, such as curcumin, resveratrol in grapes and wine, sulforaphane, and quercetin, can inhibit DNA damage repair in cancer cells, leading to cell death.

continued on page 14

Continued from page 13 — Selected Recent Publications

SHOREY LE, MADEEN EP, ATWELL LL, HO E, LÖHR CV, PEREIRA CB, **DASHWOOD RH,** and **WILLIAMS DE.** Differential modulation of dibenzo[*def,p*]chrysene transplacental carcinogenesis: Maternal diets rich in indole-3-carbinol versus sulforaphane. *Toxicol. Appl. Pharmacol*. **270**:60-69, 2013

Treating pregnant mice with the carcinogen dibenzo[*def,p*]chrysene (DBC), an environmental polycyclic aromatic hydrocarbon produced by the burning of fossil fuels, causes the offspring to develop aggressive lymphoma and lung and liver tumors. The authors showed in previous studies that supplementing the pregnant mice exposed to DBC with indole-3-carbinol (I3C), a phytochemical found in cruciferous vegetables, protected against mortality from lymphoma in the offspring and decreased the number of lung tumors. In the present study, the authors compared the effects of feeding freeze-dried broccoli sprout powder containing I3C, purified supplements of I3C, or sulforaphane (an isothiocyanate) to the pregnant mice. Surprisingly, maternal consumption of broccoli powder or sulforaphane increased morbidity in the offspring and did not inhibit tumorigenesis caused by exposure to DBC. Administering I3C with sulforaphane nullified the deleterious effects of sulforaphane alone. The sulforaphane dose in these experiments greatly exceeded the amount humans typically consume in broccoli, and the results may be explained by a sensitivity of the fetus to non-physiologically high concentrations of sulforaphane. More research is under way to understand these complex mechanisms.

HO E, BEAVER LM, WILLIAMS DE, and **DASHWOOD RH.** Dietary factors and epigenetic regulation for prostate cancer prevention. *Adv. Nutr*. **2**:497-510, 2011

The authors discuss the importance of nutrient-gene interactions in cancer, especially those involving epigenetic regulation of gene activity. DNA methylation and histone modifications affect how genes are silenced or activated. For example, most DNA in prostate cancer cells is hypo-methylated, while some specific genes are hypermethylated. This methylation activity influences how cancer progresses. The prevention or progression of prostate cancer may be influenced by dietary factors like B vitamins (folate and vitamins B_6 and B_{12}), the amino acid methionine, soy isoflavones, tea polyphenols, isothiocyanates (sulforaphane from cruciferous vegetables), selenium, and zinc, all of which influence methylation in desirable ways. Histone acetylation, an epigenetic event in which acetyl chemical groups are added to the histones that regulate DNA activity, is also associated with cancer chemoprotection. Histone deacetylase inhibitors, including fiber, garlic compounds, sulforaphane and indole-3-carbinol from cruciferous vegetables, selenium, curcumin from the spice turmeric, and tea polyphenols, prevent the removal of acetyl groups from histones, leading to apoptosis (programmed cell death) of cancer cells and the activation of tumor-suppressor genes. Much more research needs to be done, especially concerning dose, interactions, metabolism, timing, and tissue specificity.

SAUD SM, YOUNG MR, JONES-HALL YL, ILEVA L, EVBUOMWAN MO, WISE J, COLBURN NH, KIM YS, and **BOBE G.** Chemopreventive activity of plant flavonoid isorhamnetin in colorectal cancer is mediated by oncogenic Src and β-catenin. *Cancer Res*. **73**:5473-5484, 2013

Isorhamnetin is a flavonol found in onions and almonds. It is similar in structure to the flavonol quercetin and can be formed from the metabolism of quercetin in the body. An association between the consumption of dietary isorhamnetin and a reduced risk of colorectal cancer recurrence was observed in the Polyp Prevention Trial, which was a four-year, randomized, nutritional intervention study with 1,905 subjects. In the present study, mice were treated with a chemical carcinogen (azoxymethane) and a colonic irritant to cause colorectal tumors. After that treatment, groups were given diets supplemented with isorhamnetin or the flavonols quercetin, rutin, or myricetin. The mice fed the isorhamnetin-supplemented diet had fewer tumors, smaller tumors, and a much higher survival rate than the control mice. Isorhamnetin was more effective than the other flavonols and also decreased inflammation, inhibited cell proliferation, and inhibited Src oncogene activity.

MENTOR MARCEL RA, **BOBE G,** SARDO C, WANG L-S, KUO C-T, STONER G, and COLBURN NH. Plasma cytokines as potential response indicators to dietary freeze-dried black raspberries in colorectal cancer patients. *Nutr. Cancer*. **64**:820-825, 2012

Twenty-four patients with colorectal cancer were enrolled in this clinical study to determine the effect of consuming a slurry of freeze-dried black raspberry powder in water three times a day for about three weeks, while receiving no other therapy. Plasma samples and biopsies of normal and cancer tissue in the colon were taken periodically and assayed for markers of inflammation, apoptosis (programmed cell death), cell proliferation, and angiogenesis (blood vessel formation). The consumption of black raspberry slurry improved these parameters and time-dependently decreased interleukin 8, which is a cytokine (cell-signaling molecule) associated with inflammation. LPI

Developments

Michele Erickson
LPI Director of Development

Building on Pauling's Legacy

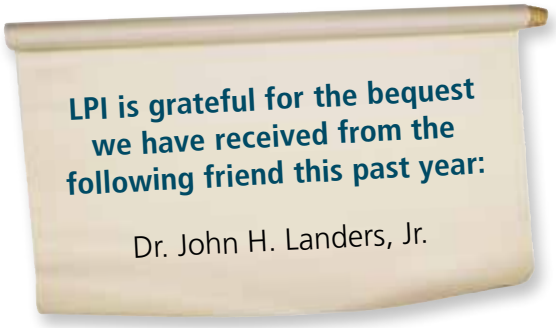
You may have noticed the honor roll of donors recognized in each LPI Research Newsletter who have made recent bequest gifts to LPI. Over the last eight years, this special group of donors has contributed more than $7,700,000 to support our research at the Linus Pauling Institute. Additionally, $2,600,000 of these gifts were used to create endowed funds that will continue supporting the work of the Institute into perpetuity. These gifts create a legacy empowering the Institute to make more ground-breaking discoveries, leverage federal research funding, and improve our ability to help people use this knowledge to live healthier lives.

The Linus Pauling Institute Legacy Circle honors loyal and generous supporters and friends who provide for the Institute in their estate plans, through wills, trusts, life-income gifts, retirement plans, life insurance policies, and other vehicles. Gifts from Linus Pauling Institute Legacy Circle members come in many forms and sizes and benefit the full range of LPI programs. What these gifts have in common is their donors' thoughtful consideration and desire to leave a legacy that enhances the Institute they hold so dear.

Fittingly named, the Linus Pauling Institute Legacy Circle gives tribute to our founder, Dr. Linus Pauling. The Legacy Circle creates an enduring commemoration honoring Dr. Pauling's life and creates a profoundly meaningful opportunity for the Institute to recognize our donors' intent to continue Dr. Pauling's mission to reduce suffering and improve human health. We know that the generosity of Linus Pauling Institute Legacy Circle members is helping to secure the future of the Linus Pauling Institute.

Membership in the Linus Pauling Institute Legacy Circle involves no dues or obligations of any kind other than an expressed intent to include the Linus Pauling Institute in your estate plans. LPI periodically holds special activities for donors, and members of the Linus Pauling Institute Legacy Circle are warmly encouraged to attend. Currently, we honor and appreciate 20 visionary donors who are Linus Pauling Institute Legacy Circle Members and whose commitment today creates opportunities for tomorrow. If you have remembered the Linus Pauling Institute in your estate planning and would like to be recognized as a Legacy Circle Member, please contact me. We would appreciate the opportunity to thank you for your generosity.

Michele Erickson
Director of Development, Linus Pauling Institute
Oregon State University Foundation
850 SW 35th Street | Corvallis, OR | 97333
Phone 541-737-3744 LPI



The **Oxygen Club of California** conference, Nutrition and Redox Biology in Development and Health, will be held May 7-10, 2014, at the University of California, Davis (http://oxyclubcalifornia.org/occ2014).

SEE US ON FACEBOOK
https://www.facebook.com/LinusPaulingInstitute

VISIT OUR NEW LPI BLOG
http://blogs.oregonstate.edu/linuspaulinginstitute

A special opportunity for friends of the Linus Pauling Institute!

Offered by the OSU Foundation, this **Estate-Planning** kit will help you:

- Set family goals and begin the estate-planning process.
- Make your will the best it can be.
- Decide if you should include a trust in your plans.
- Create a personal record book for your financial planner or attorney.

Reduce or eliminate taxes today by making a difference tomorrow for organizations that have a special place in your life–like the Linus Pauling Institute at Oregon State University!

To receive the guidebook series, simply complete our online form (http://campaignforosu.org/GuideBook), or call us at 541-737-3767. We will mail you the new estate-planning guidebooks over the next few months—complimentary and obligation-free.

Linus Pauling Institute

Stephen Lawson, Research Newsletter Editor
Oregon State University
307 Linus Pauling Science Center
Corvallis, Oregon 97331

phone: 541-737-5075
fax: 541-737-5077
email: lpi@oregonstate.edu
Web site: http://lpi.oregonstate.edu

Non-Profit Org.
U.S. Postage
PAID
Portland, OR
Permit No. 1006

Look for these informative articles inside!

Special thanks to Barbara McVicar for editorial assistance and photographs, authors of signed articles, and Dick Willoughby for the logo photograph of Linus Pauling.

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.

Printed on recyclable paper using soy inks