



OREGON STATE UNIVERSITY

The Linus Pauling Institute

RESEARCH NEWSLETTER



From the Director

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

Ever since the Linus Pauling Institute of Science and Medicine moved from Palo Alto, California, to Corvallis, Oregon, to become the Linus Pauling Institute at Oregon State University, one of our highest priorities has been to build a state-of-the-art research facility to house the Institute and serve as a high-profile working memorial for Linus Pauling. Indeed, the Memorandum of Understanding signed in 1996 between the University and the Institute stated that "OSU and LPISM acknowledge that a new building with modern facilities would be highly desirable. Construction of such a building will be contingent upon success in developing private gifts and other funding." Now, 11 years later, it gives me great pleasure to announce to you that OSU and LPI have been successful in securing these "private gifts and other funding" and that the **Linus Pauling Science Center** is scheduled to open its doors on the campus of Oregon State University in 2010!

This building is the centerpiece of the University's first-ever capital campaign and one of its major construction projects. Under the outstanding leadership of OSU's president, Dr. Edward Ray, and the president and CEO of the Oregon State University Foundation, Mike Goodwin, we have been able to raise a total of \$62.5 million for the building. All of us at LPI are extremely grateful to Dr. Ray and his leadership team for their unwavering support of the Institute and their tireless—and highly successful—efforts to raise the necessary funds for the building. Dr. Ray believes that "preventive health care is the future of medicine," and he has made "realizing fundamental contributions in the life sciences and optimizing the health and well-being of the public" one of the University's strategic goals for the 21st century.

The lead gift for the building of \$20 million was provided by the Wayne and Gladys Valley Foundation in Oakland, California, in March of this year, and shortly thereafter was followed by a \$10.65 million gift from Al and Pat Reser,

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Diet and Optimum Health Conference

*Stephen Lawson
LPI Administrative Officer*

The fourth LPI Diet and Optimum Health Conference, co-sponsored by the Oxygen Club of California (OCC) and Oregon Health & Science University, convened in Portland from May 16th to 19th. The conference featured 22 speakers from around the world and was organized into seven sessions: *Flavonoid Functions; New Discoveries of Vitamin C in Health and Disease; Lipoic Acid: Biological Mechanisms of Action; Neuroprotection by Antioxidants; Maternal Diet, Genes, and Epigenetics; Cancer Chemoprevention; and Obesity, Exercise, and Gene Regulation.*

The conference opened on Wednesday evening with a special session of oral presentations culled from the many abstracts submitted for posters. Additionally, finalists for the Young Investigator Awards, sponsored jointly by LPI and OCC, gave short presentations. Anna Hsu, Jeff Monette, and Keith Nylin were selected for Awards, which were presented by Dr. Maret Traber of LPI and Dr. Lester Packer of OCC at the banquet on Friday evening.

Flavonoid Functions

Chaired by Jeff Blumberg (Tufts)

- **Gary Williamson** (Nestlé Research Center, Switzerland) gave an overview of the role of flavonoids in human health. Catechins—flavonoids from tea—affect energy metabolism, and isoflavones from soy improve bone mineral density. Sugars affect the activity and metabolism of ingested flavonoids, which are poorly absorbed. Flavonoids may lower the risk of chronic diseases, especially heart disease and inflammatory diseases.
- **Augustin Scalbert** (Centre de Recherche de Clermont-Ferrand/Theix, France) discussed the comprehensive database of flavonoids that his group is developing. The database provides the flavonoid content of foods and analytical methods. Using a "metabolomic" approach, metabolites of flavonoids have been identified in urine that will be valuable in nutritional epidemiological studies.
- According to

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— Designated by the National Institutes of Health as a —

Center of Excellence for Research on Complementary and Alternative Medicine



Continued from cover — From the Director

1960 graduates of OSU and owners of Reser's Fine Foods. The Valley Foundation has been very generous to OSU in the past and has provided many other significant gifts to the University. The Resers also are exceptionally generous philanthropists who have supported the University for many years. With their gift of over \$10 million for the Linus Pauling Science Center, the Resers have shown their strong commitment to the academic programs at OSU, for which we are extremely grateful.

The gifts from the Valley Foundation and the Resers, together with significant contributions from a select few other donors, brought the philanthropic total to \$31.25 million, half of the projected construction costs. With these commitments in hand, the University was able to request matching funds from the State of Oregon, which were approved by the legislature in July. Thus, the State of Oregon will provide \$31.25 million from bond proceeds in March of 2009 towards construction of the Linus Pauling Science Center.

The gifts from the Valley Foundation and the Resers and Oregon's matching funds are truly transformational for OSU and LPI. One of the biggest challenges that the Institute has faced for several years is the lack of high-quality research space. Upon completion of the Linus Pauling Science Center, the Institute will have a new, permanent home that brings together all of our principal investigators for the first time in state-of-the-art research space. For me, this is a dream come true that gives me great pride and satisfaction as director of LPI. This building undoubtedly represents a milestone in the Institute's history that will elevate our research to the next level and establish LPI and OSU as one of the premier institutions in the world for cutting-edge research on diet, micronutrients, and health.

Half of the approximately 120,000-sq. ft. building will be occupied by LPI; the other half will provide teaching and research space for the Department of Chemistry. Hence, the Center will honor Dr. Linus Pauling, our founder and OSU's most distinguished alumnus, by continuing his legacy in orthomolecular medicine and chemistry. The building will be strategically located on campus proximate to relevant departments and research centers and provide a focus for OSU's health and life sciences research. In this way, the Center will create a cohesive interdisciplinary research environment that fosters daily interactions and collaborations among the Institute's scientists and those in chemistry and other departments at OSU, spawning new ideas for research

projects and joint grant applications. In addition, the Center will allow expansion of the Institute from its current 11 laboratories to about 15.

With the funding for the building secured, we are now seeking gifts to support the expansion of our research program, which will be focused on a new initiative called the Healthy Aging Program. The goal of this program is to help everyone achieve their maximum healthspan through optimum health—we want people to live better, not just longer. Dr. Tory Hagen, our expert in the biology of aging, said it best: "Aging is not a death sentence; aging is a normal part of life. Successful aging is when a person can continue to lead a healthy and active life even with the normal biological decline that comes with aging."

Over the last few years, discoveries in the fields of immunosenescence (age-related dysregulation of immune

function that contributes to increased susceptibility to infection, cancer, and autoimmune diseases) and epigenetics (heritable changes in gene function that occur without a change in the underlying DNA sequence) have revolutionized our understanding of how we age and why some people age more gracefully than others. These remarkable new advances, coupled with the Institute's expertise in age-essential micro-nutrients, antioxidants,

and gene-nutrient interactions, have come together in an extraordinary manner like the pieces of a puzzle to present us with a unique opportunity to look at the biology of aging from a new perspective.

As part of this new initiative, the Institute is planning to hire several faculty and establish new research laboratories in the areas of immunosenescence, epigenetics, and neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease. These laboratories will be housed in the new building and constitute LPI's Healthy Aging Program, which will be directed by Dr. Hagen. This is a big undertaking, and we will only be successful with your continued support.

LPI's future looks extremely bright, with the construction of a modern research building starting soon and the launch of the Healthy Aging Program. I look forward to leading the Institute through the building process in the coming years, both as it relates to the Linus Pauling Science Center and the expansion of the Institute's research scope. I hope you share my excitement and the excitement of everyone here at LPI over these new developments and invite you to join us at OSU for the Center's grand opening in 2010! **LPI**



A possible design for the Linus Pauling Science Center—the grand opening is scheduled for 2010

William Helferich (University of Illinois-Champaign/Urbana), Americans are experiencing “isoflavone creep” because many food products are supplemented with soy isoflavones. Isolated isoflavones like genistein or genistin have been found to stimulate breast cancer in animals and to inhibit the effect of the breast cancer drug tamoxifen. On the other hand, soy flour did not enhance tumor growth, suggesting that the profile of components in soy is an important factor.

New Discoveries of Vitamin C in Health and Disease

Chaired by **Brigitte Winklhofer-Roob** (University of Graz, Austria) and **Enrique Cadenas** (USC)

- **Fred Stevens** (LPI) has been elucidating reactions between oxidized fat and vitamin C. In test tube and cell culture experiments, vitamin C (ascorbic acid) reacts with oxidized lipids to ultimately form conjugates, a process that presumably protects biomolecules like DNA from damage. Ascorbylated acrolein (an aldehyde formed from oxidized fat) has been detected in human urine, suggesting that ascorbylation (reaction with vitamin C) of reactive aldehydes may be important physiologically.
- Over 35 years ago, Cameron and Pauling began investigating the use of high-dose vitamin C as cancer therapy. **Mark Levine** (NIH) has contributed significantly to our understanding of how vitamin C kills cancer cells. He found that high-dose intravenous vitamin C produces hydrogen peroxide in the extracellular milieu (but not in blood), which selectively kills cancer cells *in vitro* through apoptosis (programmed cell death) and necrosis.
- **James May** (Vanderbilt) emphasized the important role of vitamin C in neuronal function and protection. The brain has among the highest concentrations of vitamin C of any organ in the body. Oxidized vitamin C can enter the brain through cellular glucose transporters, but the sodium-dependent vitamin C transporter 2 in cell membranes is responsible for vitamin C's access to neuronal tissue, where vitamin C protects against oxidative damage implicated in neurodegenerative diseases.

Lipoic Acid: Biological Mechanisms of Action

Chaired by **Lester Packer** (USC) and **Kate Shay** (LPI)

- **Tory Hagen** (LPI) discussed work showing that feeding old rats (*R*)-alpha-lipoic acid reversed the age-related decline in glutathione levels in liver cells. Glutathione is an antioxidant and plays an important role in detoxification, and its increase in liver cells improved resistance to toxins in the old rats. Lipoic acid affects the regulation of cell-signaling molecules that control the expression of the Antioxidant Response Element genes in DNA, which provide protection against toxins, oxidants, and mutagens.
- **Erik Henriksen** (University of Arizona) noted that exercise and lipoic acid improve insulin resistance in skeletal muscles of patients with type 2 diabetes. Insulin resistance is characterized by increased oxidative stress, which is attenuated by exercise and lipoic acid. Lipoic acid also improves glucose tolerance, lowers triglycerides, and reduces the amount of protein damage as measured by protein carbonyls.
- A mouse model of multiple sclerosis (experimental autoimmune encephalomyelitis, EAE)

has allowed investigators to study the therapeutic effect of lipoic acid. **Dennis Bourdette** (Oregon Health & Science University) explained that lipoic acid suppresses EAE in mice—reducing paralysis and inhibiting inflammation. In a short human trial, lipoic acid at high doses found to be effective in the mouse model decreased a marker of inflammation in MS patients, although the molecular mechanisms for the effect remain obscure.

Neuroprotection by Antioxidants

Chaired by **Joseph Quinn** (OHSU) and **Joe Beckman** (LPI)

- **Raymond Burk** (Vanderbilt) has studied the effect of antioxidant vitamin deficiencies on the central nervous system in guinea pigs.

Weanling guinea pigs deprived of both vitamins, but neither singly, developed severe neurological problems, including paralysis. F₂ isoprostanes—markers of lipid oxidation—were increased in the



(left to right) *Qi Chen, Mark Levine, Balz Frei, and Yao Hui Wang*

vitamin-deprived animals. The brains of mice without a selenium transport protein had low levels of selenium, leading to severe neurological problems. Additionally, combined selenium and vitamin E deficiency caused liver damage in rats.- **Chandan Sen** (Ohio State University) presented evidence that alpha-tocotrienol, one of the eight members of the vitamin E family, protects neural cells in culture from glutamate toxicity. He also discussed results showing that orally supplemented alpha-tocotrienol ameliorated brain damage in hypertensive rats induced by strokes.
- **Alberto Ascherio** (Harvard) discussed results from epidemiological studies investigating the relationship between dietary intake of antioxidants and the risk of neurological disease. Two long-term, large-scale epidemiological studies did not find any protective effect of dietary or supplemental vitamins C and E in reducing the risk of Parkinson's disease (PD), even though oxidative stress plays a role in the disease. Several studies found that urate, formed in the body from dietary precursors, decreased the risk of PD, especially in men. Additionally, the long-term use of vitamin E supplements was associated with a reduced risk of ALS.

Maternal Diet, Genes, and Epigenetics

Chaired by **Carroll Cross** (University of California-Davis) and **Tammy Bray** (LPI)

- **Randy Jirtle** (Duke) discussed how maternal nutrition affects epigenetics and disease risk in offspring. Using mice, he showed that maternal supplementation with B vitamins or genistein from soy affected coat color and risk of obesity, diabetes, and cancer in offspring. Nutrition did not affect the genes in DNA, but rather influenced their expression and chemical modifications of their protein products. For example, many children born to mothers who endured the Dutch famine of 1944-5 developed health

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(left to right) Mark Levine, OSU President Ed Ray, and Tammy Bray

and mental problems as adults. • **Dave Williams** (LPI) noted that leukemia and lymphoma are the most common childhood cancers. In mice, maternal exposure to a carcinogenic polycyclic aromatic hydrocarbon called dibenzo[a,l]pyrene causes lymphoma in offspring. The offspring are substantially protected against cancer if indole-3-carbinol, a component of cruciferous vegetables; caffeinated green tea; caffeine; or chlorophyllin, a derivative of chlorophyll, is added to the maternal diet.

• Choline is an essential nutrient that must be obtained dietarily, primarily from milk, eggs, wheat germ, and liver in the form of phosphatidylcholine, or lecithin. **Steven Zeisel** (UNC) discussed how choline in the maternal diet affects the development of brain structure and function in the fetus. Fetal choline intake also affects adult memory, although supplemental choline in adults does not significantly affect memory function. Choline deficiency in adults can cause muscle and liver damage.

Cancer Chemoprevention

Chaired by **Rod Dashwood** (LPI)

• **Emily Ho** (LPI) described work with sulforaphane, an isothiocyanate found in cruciferous vegetables, especially broccoli. In human prostate cancer cells in culture and implanted in mice, sulforaphane apparently acts as a histone deacetylase (HDAC) inhibitor, which results in anticancer effects by affecting how proteins called histones surround DNA. HDACs are overexpressed in cancer cells, thereby silencing tumor suppressor genes. In humans, sulforaphane in broccoli sprouts inhibited HDAC activity in blood cells.

• Glucuronidation is a metabolic process in which compounds are chemically modified for excretion from the body. **Johanna Lampe** (University of Washington) explained that phytochemicals in cruciferous vegetables, soy, and citrus fruit increase glucuronidation of carcinogens and, possibly, some drugs. Most studies to date have been done using animals; few human data are available. • **Young-Soon Surh** (Seoul National University, Korea) discussed the role of inflammation in cancer and how certain phytochemicals, such as curcumin in tumeric, ginger, and sulforaphane in cruciferous vegetables, may help protect against cancer by influencing anti-inflammatory pathways. Dietary antioxidants protect against cancer in part by activating cell signals that affect detoxification genes.

Obesity, Exercise, and Gene Regulation

Chaired by **Urszula Iwaniec** (OSU)

• **Jacqueline Van Hooissen** (University of Portland, Oregon) discussed the effect of physical exercise on mood and neuronal function. Exercise improves cerebral blood volume, which protects against damage caused by pathological events

in the brain. It also results in neurogenesis, increased synthesis of neuropeptides, and changes in brain morphology. Animal experiments have shown that chronic exercise affects regions of the brain different from those affected by acute exercise, and research is under way to understand how those changes affect behavior. • **Kelvin Davies** (USC) noted that endurance training and sprint training result in different biochemical adaptations. There is a much larger increase in muscle mitochondria—cellular organelles responsible for energy production—and endurance capacity after endurance training. Intense exercise also generates free radicals, and vitamin E deficiency during intense exercise causes damage to muscle mitochondria. • **Donald Ingram** (Louisiana State University) addressed the effects of caloric restriction (CR) on aging. In rodents, CR reduces body fat, lowers body temperature, inhibits tumor formation, reduces fasting glucose levels, attenuates age-related decline, improves neuronal function, and increases protection against toxins. CR has been shown to extend lifespan in short-lived species, but this has not yet been observed in higher primates, including humans.

• **Maria Luz Fernandez** (University of Connecticut) presented the results of several weight-loss studies. After ten weeks on a low-calorie diet and with increased physical activity, overweight women had reduced weight, abdominal fat, insulin resistance, LDL cholesterol levels, and triglyceride levels. Additionally, metabolic syndrome was significantly attenuated. In another short study, overweight men were given a carbohydrate-restricted diet supplemented with soluble fiber. After twelve weeks, the men had reductions in weight, abdominal fat, triglyceride levels and blood pressure, while HDL (“good”) cholesterol levels increased.



(left to right) Barbara McVicar and Tracy Oddson

Public Session

A session on Saturday, open to the public, featured *Washington Post* columnist Sally Squires, whose presentation, *Secrets of the Lean Plate Club: Tales from the Waistline Wars*, focused on strategies for maintaining optimal weight. Ms. Squires noted that about one billion people worldwide are overweight and that by 2020, 75% of mortality from chronic disease will be weight related. She discussed the alarming trends in ever-enlarging portion size, or “calorie creep” that, when combined with physical inactivity, result in unhealthy weight gain. The Lean Plate Club has six goals: 1) eat smart, 2) move more, 3) add healthy habits, 4) reach a healthier weight, 5) adopt a positive approach, and 6) ban the word “don’t”. Ms. Squires emphasized the need for about 30 minutes of daily physical activity—not necessarily exercise—for health and 60-90 minutes of daily activity to lose weight. She described a number of simple steps to attain better health: eat a variety of foods, including fish, beans, whole grains, fruit and vegetables; get enough sleep; eat breakfast; drink plenty of water; take a calcium supplement with vitamin D; if over 50, take a vitamin B12 supplement; and achieve caloric balance between intake and expenditure. **LPI**

Linus Pauling Institute Prize for Health Research



Dr. Mark Levine

The 2007 LPI Prize for Health Research, consisting of a medal and \$50,000, was awarded at the Diet and Optimum Health Conference on May 18th to Mark Levine, M.D. Dr. Levine is Chief of the Molecular and Clinical Nutrition Section of the Digestive Diseases Branch of the National Institute of Diabetes & Digestive & Kidney Diseases in Bethesda, Maryland. He earned his B.A. degree in biology at Brandeis University and his M.D. degree at Harvard Medical School. He has been at the NIH since 1980, conducting clinical research and seeing patients, and has published over 125 scientific papers. Dr. Levine joins Drs. Bruce Ames (2001), Walter Willett (2003), and Paul Talalay (2005) as recipients of the LPI Prize.

Dr. Levine became interested in research while in medical school. When he learned that the conversion of dopamine to norepinephrine is dependent on vitamin C, he became particularly interested in vitamin C and was shocked to find that its RDA was based solely on the prevention of scurvy, not on function or optimum levels. For many years, Dr. Levine has worked on the pharmacokinetics of vitamin C—how much of different doses is absorbed into the blood from the gastrointestinal tract and how that influences blood levels and excretion. He has also carefully documented differences in blood concentrations of vitamin C following oral or intravenous infusion, finding that such concentrations are 70 times greater after intravenous infusion. These observations have rekindled interest in the therapeutic use of vitamin C in cancer. Dr. Ewan Cameron, Linus Pauling's clinical collaborator in vitamin C and cancer studies, began treating terminal cancer patients with high-dose intravenous and oral vitamin C in 1971 and reported favorable responses. The infamous Mayo Clinic studies in the 1970s and 1980s failed to replicate Cameron's work but used only oral vitamin C. Consequently, vitamin C in the blood of those patients was unlikely to have attained sufficient concentrations to kill cancer cells.

Dr. Levine's recent papers have described elegant studies on the molecular mechanism responsible for the cancer cell toxicity of vitamin C. He and his co-workers found that high concentrations of vitamin C in the extracellular fluid—but not in blood—attained by intravenous infusion, generate the ascorbate free radical, which then stimulates the formation of hydrogen peroxide. Hydrogen peroxide then diffuses

into cancer cells and kills them via several mechanisms, including the depletion of ATP—a molecule critical in cellular energy—in the mitochondria. In the anaerobic metabolism of cancer cells, mitochondria may be especially sensitive to hydrogen peroxide, whereas mitochondria in normal cells exhibiting aerobic metabolism are not. Dr. Levine found that cancer cells exposed to high concentrations of vitamin C die by necrosis or apoptosis (programmed cell death), whereas normal cells are unaffected. Dr. Levine believes that the anticancer mechanism elucidated for vitamin C may also be useful in treating viral and bacterial infections.

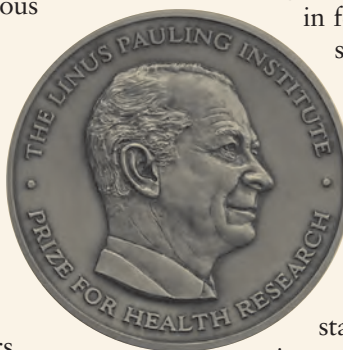


Dr. Levine gives his plenary lecture on vitamin C

Dr. Levine's pharmacokinetic studies in healthy young men and women led to the observation that circulating cells in blood reach their highest concentration of vitamin C after a dose of about 400 mg, which also results in near maximum plasma concentrations. However, vitamin C is excreted fairly rapidly, and Dr. Levine's recent pharmacokinetic model proposes that the concentration in blood can be maintained at its maximum by taking vitamin C several times a day.

Dr. Levine has also studied the transport of vitamin C into cells. He characterized one of the vitamin C transporters that gets the vitamin into the brain and lung and discovered the critical role of vitamin C in fetal development. His vitamin C transport studies also found that flavonoids, a class of polyphenolic phytochemicals found in fruit and vegetables, inhibit the absorption of vitamin C.

As one nomination letter noted, "Without a doubt, Dr. Levine is one of the leading scientists in the fields of vitamin research." Another nominator stated that "his work has had such a major impact that he is truly considered the world's expert on vitamin C and its potential for treatment of disease. Thus, he has provided solid justification for... Linus Pauling's proposals for the use of vitamin C against cancer and infections." **LPI**





Is Coffee Harmful or Healthful?

Victoria J. Drake, Ph.D.
LPI Research Associate

Coffee is an infusion of ground, roasted coffee beans and is one of the most widely consumed beverages in the world. The drink contains more than a thousand different components, including carbohydrates, lipids, vitamins, minerals, alkaloids, phenols, and nitrogenous compounds. Overall, there is little evidence of health risks and some evidence of health benefits for adults consuming moderate amounts of coffee (3-4 cups/day, providing 300-400 mg/day of caffeine). Beneficial effects on mental state, along with coffee's aroma and flavor, are likely reasons why coffee is such a popular beverage. Because of the many variations in the manner in which coffee is prepared and consumed, it is difficult to interpret epidemiological studies.

Compounds in Coffee That May Affect Human Health

Caffeine

Caffeine (1,3,7-trimethylxanthine) is an alkaloid that occurs naturally in coffee beans. Caffeine consumption has been linked to several positive effects on mental state, including increased alertness, elevated mood, decreased fatigue, and improved work performance. Moreover, two prospective epidemiological studies have reported that moderate coffee drinking significantly decreases the risk of suicide. While the specific mechanisms for effects on health and behavior are not established, it is known that caffeine functions by blocking two adenosine receptors (A_1 and A_{2A}) in the brain and consequently has an overall stimulatory effect on the central nervous system.

Diterpenes

Boiled coffee beans also contain significant amounts of the two diterpenes (a class of compounds with four isoprene units), cafestol and kahweol. Some studies have associated these compounds with increased levels of LDL (the "bad" cholesterol) and total cholesterol. Filtered coffee, on the

other hand, contains very low levels of cafestol and kahweol because they are mostly removed by the paper filter during brewing. Indeed, filtered coffee consumption does not appear to elevate serum cholesterol concentrations.

Chlorogenic acid

Coffee naturally contains polyphenolic compounds called chlorogenic acids, which are esters of quinic and cinnamic acids. The most prevalent individual chlorogenic acid is 5-O-caffeoylquinic acid but often simply called chlorogenic acid. Chlorogenic acid has been shown to exhibit antioxidant activity *in vitro*; however, the extent of antioxidant activity *in vivo* is unclear.

Other components

Coffee contains a number of other compounds that could potentially affect human health, such as various micronutrients like magnesium, potassium, niacin, and vitamin E. Additionally, coffee is a dietary source of several lignans (phytoestrogens) that may alter glucose metabolism as well as trigonelline (*N*-methylnicotinic acid), a phytochemical that has been shown to have a hypoglycemic effect in diabetic rats.

Potential Health Benefits of Coffee Consumption

Prevention of type 2 diabetes mellitus

Several prospective epidemiological studies have consistently reported that habitual intake of caffeinated coffee lowers the risk of type 2 diabetes mellitus (DM). Interestingly, decaffeinated coffee was recently reported to decrease type 2 DM risk and also to positively alter glucose metabolism, suggesting that non-caffeine components in coffee may offer protection against the disease. More prospective research is needed to determine whether consumption of decaffeinated coffee also lowers type 2 DM risk. Additionally, mechanistic studies investigating the effects of individual constituents of coffee will provide insight into the observed anti-diabetic effect.

Prevention of Parkinson's disease

To date, results of most case-control and prospective cohort studies suggest that routine coffee and caffeine consumption decreases the risk of Parkinson's disease in men but not in women. However, one study in 77,713 women participating in the Nurses' Health Study found that coffee consumption decreased Parkinson's disease risk in women who had never used postmenopausal estrogen, and high coffee intakes actually increased risk of Parkinson's disease in women using estrogen replacement therapy. More prospective research is necessary to determine whether coffee or caffeine intake alters Parkinson's disease risk and whether postmenopausal use of estrogen modifies the association.

Prevention of liver diseases

Some studies have found that coffee intake decreases risk of liver diseases, including cirrhosis and hepatocellular carcinoma. Caffeine and chlorogenic acid have been reported to inhibit hepatic carcinogenesis in animals, yet more mechanistic studies are necessary to determine the exact relationship.



Prevention of colorectal cancer

Case-control studies have generally found that coffee drinking lowers risk of colorectal cancer; however, prospective cohort studies have largely reported no effect. It is generally recognized that prospective studies in which nutrient intake is measured in healthy people and disease outcome is determined later have a stronger experimental design than case-control studies, which rely on comparing subjects diagnosed with disease to other subjects without the disease. Thus, more prospective research on coffee consumption and colorectal cancer is warranted to make any definitive conclusions about the association.

Effects on mental state

Coffee intake has been shown to improve overall mood and mental performance; however, these effects are highly dependent on caffeine dose and habituation. Negative effects, such as increased anxiety and sleep disturbances, have also been reported with coffee intake, especially in non-habitual consumers.

Potential Health Risks of Coffee Consumption

Cardiovascular diseases

Case-control and prospective epidemiological studies on the effects of coffee intake on cardiovascular disease report conflicting results. Therefore, it is not clear whether coffee drinking increases risk for various cardiovascular diseases, including myocardial infarction, stroke, and coronary heart disease. However, studies have associated consumption of caffeinated coffee with increases in blood pressure and plasma homocysteine levels, two independent risk factors for cardiovascular diseases.

- **Blood pressure**

It is well-established that acute caffeine administration increases blood pressure, but recent studies suggest that chronic consumption of caffeinated coffee may not raise blood pressure, presumably because tolerance to caffeine develops.

- **Plasma total homocysteine**

Some studies have found that coffee drinking increases plasma total homocysteine; however, it is not clear whether such changes translate to an overall increase in risk for cardiovascular diseases.

- **Cardiac arrhythmias**

While two case reports have linked caffeine toxicity to atrial and ventricular arrhythmias, most epidemiological studies have found no association between coffee consumption and cardiac arrhythmias.

Cancer

Most studies to date report that moderate coffee drinking does not elevate risk for pancreatic, lung, bladder, or renal cancer.

Osteoporosis and bone fracture

Studies investigating the effect of coffee or caffeine consumption on osteoporosis and bone fracture risk have reported mixed results. While large-scale, prospective studies are needed to determine whether coffee or caffeine intake

alters osteoporosis or fracture risk, currently available data suggest that limiting coffee consumption to three cups daily (300 mg/d of caffeine) and ensuring adequate calcium and vitamin D nutrition may help prevent any adverse effects on bone status.

Caffeine and pregnancy complications

Some studies have reported that high intakes of caffeine during pregnancy are associated with adverse effects, including spontaneous abortion, retarded fetal growth, and low birth weight. Studies suggest that caffeine does not influence preterm delivery or birth defects. Currently, it is recommended that pregnant women limit daily caffeine intake to 300 mg (about three cups of coffee).

Special risk groups

In addition to pregnant women, older adults may be more vulnerable to any adverse effects of caffeine, including acute increases in blood pressure and any negative effects on bone status. Caffeine distributes primarily to lean body mass instead of adipose tissue, and older adults have lower lean mass to adipose ratios than younger adults; thus, the caffeine dose may be more concentrated in older individuals. Another group that may be susceptible to the negative effects of caffeine is children, but studies in this age group are limited, and additionally, most studies have focused on behavioral endpoints. High caffeine intakes in children have been linked to increased anxiety and sleep disturbances.

For more information on coffee and health, please see the Linus Pauling Institute's Micronutrient Information Center (<http://lpi.oregonstate.edu/infocenter/foods/coffee>).

LPI

LPI Pilot Project Grants

The LPI Pilot Project Grants are designed to stimulate innovative research projects by Oregon State University scientists that are relevant to the mission of the Institute. These \$25,000, one-year awards, made possible by financial support from LPI donors, enable investigators to obtain preliminary results that can be used to support research grant applications to federal funding agencies for more extensive projects.

Three proposals were funded in 2007:

- **Regis Moreau** of LPI for "Down-regulation of triglyceride synthesis by (*R*)-alpha-lipoic acid"
- **Siva Kolluri** of the Department of Environmental and Molecular Toxicology for "Screen for dietary bioactives that convert Bcl-2 from a protector to a killer"
- **Hiroko Dodge** of the Department of Public Health for "Dietary supplement usage and plasma vitamin E and C levels among the Okinawan oldest old"

Please look for articles about these projects in future LPI Research Newsletters.



Recent Publications by LPI Scientists

Selected summaries by Stephen Lawson

A comprehensive list of all LPI scientific papers published in 2006 and 2007 appears on pages 13 and 14.

*Summaries of some representative papers appear below. LPI scientists are identified in **boldface**.*

Cancer Chemoprotection

COPE RB, LOEHR C, **DASHWOOD R**, and KERKVLIT N. Ultraviolet radiation-induced non-melanoma skin cancer in the Crl:SHK:hr-1 hairless mouse: augmentation of tumor multiplicity by chlorophyllin and protection by indole-3-carbinol. *Photochem. Photobiol. Sci.* 5:499-507, 2006.

The authors conducted experiments to determine the effect of dietary supplementation with high amounts of chlorophyllin (a derivative of chlorophyll) or indole-3-carbinol (I3C, a phytochemical in cruciferous vegetables) on non-melanoma skin cancer in hairless mice subjected to ultraviolet radiation. Mice supplemented with chlorophyllin exhibited more tumors than controls, whereas supplementation with I3C decreased tumor multiplicity. Neither treatment affected tumor incidence or immunosensitivity. The molecular mechanisms responsible for the observations remain obscure.

COLGATE EC, **MIRANDA CL**, **STEVENS JF**, **BRAY TM**, and **HO E**. Xanthohumol, a prenylflavonoid derived from hops, induces apoptosis and inhibits NF-kappaB activation in prostate epithelial cells. *Cancer Lett.* 246:201-209, 2006.

Xanthohumol is the major flavonoid in the hops used to make beer and has been shown to possess anticancer properties by inhibiting all stages of carcinogenesis (initiation, promotion, and progression). Therefore, the authors added xanthohumol or its oxidation product to cultured human non-malignant hyperplastic prostate cells and malignant prostate cells. Both compounds decreased cell viability in a dose-dependent manner and induced apoptosis (programmed cell death). In hyperplastic prostate cells, xanthohumol and its derivative substantially decreased NF-kappaB activity. NF-kappaB acts as a "pro-survival" factor, thereby inhibiting apoptosis. The effective dose of xanthohumol probably cannot be obtained by drinking beer. Animal studies have not demonstrated any toxicity of high doses of xanthohumol, but human studies are needed before xanthohumol can be recommended as a dietary supplement.

HIGDON JV, **DELAGE B**, **WILLIAMS DE**, and **DASHWOOD RH**. Cruciferous vegetables and human cancer risk: epidemiologic evidence and mechanistic basis. *Pharmaco. Res.* 55:224-236, 2007.

In this review article, the authors examined the evidence for a protective effect against cancer for the consumption of cruciferous vegetables, such as broccoli, Brussels sprouts, cabbage, cauliflower, turnips, collard greens, and kale. These vegetables contain phytochemicals called glucosinolates that produce isothiocyanates (e. g., sulforaphane) and indole-3-carbinol when chewed or chopped. Epidemiological evidence for the prevention of lung cancer, colorectal cancer, breast cancer, and prostate cancer has been inconsistent, suggesting that genetic variations, or polymorphisms, in how the glucosinolates are metabolized may influence their protective effects. For example, people who eliminate the compounds

slowly may exhibit more protection against cancer.

Additionally, cooking may inactivate myrosinase, an enzyme released during chopping or chewing that produces the active phytochemicals, resulting in diminished bioavailability. Boiling leeches glucosinolates from vegetables, and as much as 60% may be lost by boiling for 15 minutes.

YU Z, **MAHADEVAN B**, **LOEHR CV**, **FISCHER KA**, **LOUDERBACK MA**, **KRUEGER SK**, **PEREIRA CB**, **ALBERSHARDT DJ**, **BAIRD WM**, **BAILEY GS**, and **WILLIAMS DE**. Indole-3-carbinol in the maternal diet provides chemoprotection for the fetus against transplacental carcinogenesis by the polycyclic aromatic hydrocarbon dibenzo[*a,l*]pyrene. *Carcinogenesis* 27:2116-2123, 2006.

Polycyclic aromatic hydrocarbons (PAHs), formed by the combustion of organic material like coal, cigarettes, cooking oil, and wood, are environmental carcinogens. Maternal exposure to PAHs (transplacental carcinogenesis) has been implicated in childhood and adult cancers, including leukemias and lymphoma. The authors supplemented pregnant mice exposed to one PAH called dibenzo[*a,l*]pyrene (DBP) with indole-3-carbinol (I3C), a phytochemical in cruciferous vegetables that protects against cancer. They found that maternal supplementation with I3C significantly reduced mortality due to aggressive lymphoma in offspring and decreased lung tumor multiplicity in offspring that survived ten months after birth.

PRATT MM, **REDDY AP**, **HENDRICKS JD**, **PEREIRA C**, **KENSLER TW**, and **BAILEY GS**. The importance of carcinogen dose in chemoprevention studies: quantitative interrelationships between dibenzo[*a,l*]pyrene dose, chlorophyllin dose, target organ DNA adduct biomarkers, and final tumor outcome. *Carcinogenesis* 28:611-624, 2006.

In a study with over 12,000 trout, the authors determined dose-response curves for a carcinogen and a chemoprotective compound. The carcinogen dibenzo[*a,l*]pyrene (DBP)—a polycyclic aromatic hydrocarbon formed from the combustion of organic material—caused a dose-dependent formation of tumors in the liver, stomach, and swim bladder. Co-administration of chlorophyllin—a derivative of natural chlorophyll—dose-dependently inhibited tumor formation except in the liver at the highest dose of DBP. At that dose, much higher concentrations of chlorophyllin were required for tumor inhibition. These results emphasize the need to choose the experimental carcinogen dose carefully, since the choice of a very high dose might lead to the erroneous conclusion that the putative chemoprotective compound is ineffective. In other words, one cannot extrapolate from high dose-response relationships often selected for experimental studies to low dose-response relationships frequently encountered in human carcinogen exposure.

MYZAK MC, **Tong P**, **DASHWOOD WM**, **DASHWOOD RH**, and **HO E**. Sulforaphane retards the growth of human PC-3 xenografts and inhibits HDAC activity in human subjects. *Exp. Biol. Med.* 232:227-234, 2007.

Sulforaphane is an isothiocyanate found in cruciferous vegetables, such as broccoli, Brussels sprouts, cauliflower, and cabbage. Broccoli sprouts contain particularly high amounts of sulforaphane, which has been identified as an anticarcinogen based on its induction of Phase 2 liver enzymes involved in the detoxification and metabolism of carcinogens. The authors have identified another anticancer role of sulforaphane: the inhibition of histone deacetylase (HDAC). DNA is wrapped around proteins called histones, and changes in the conformation of histones determine when genes are turned on or off. When acetyl groups are added to histones, genes are turned on, and removal of the acetyl groups inactivates genes. HDAC removes acetyl groups from histones, which results in gene inactivation. In cancer cells, this process inactivates tumor suppressor genes. If HDAC could be inhibited, then the tumor suppressor genes could be turned back on to block cancerous cell growth. In the present study, the authors found that feeding a sulforaphane-supplemented diet to mice implanted with human prostate cancer cells significantly inhibited tumor growth. HDAC activity was also suppressed. When healthy humans were given a dose of sulforaphane as broccoli sprouts, HDAC activity in peripheral blood mononuclear cells was significantly inhibited. Each subject consumed about 105 mg of sulforaphane in the 68-gram serving (about one cup) of broccoli sprouts. An equivalent amount of sulforaphane is present in about 570 grams of mature broccoli.

Heart Disease

WIDLANSKY ME, DUFFY SJ, HAMBURG NM, GOKCE N, WARDEN BA, WISEMAN S, KEANEY JF JR, FREI B, and VITA JA. Effects of black tea consumption on plasma catechins and markers of oxidative stress and inflammation in patients with coronary artery disease. *Free Rad. Biol. Med.* 38:499-506, 2005.

The authors previously reported that consumption of black tea improved endothelial function in patients with heart disease, as measured by brachial artery blood flow. In this study, the authors measured catechins from tea in the plasma of those subjects to determine which may have been responsible for the beneficial effect. Individual catechins did not affect plasma antioxidant status, endothelial function, or C-reactive protein (a marker of inflammation), suggesting that other flavonoids in tea and the diet are responsible for the observed improvement in endothelial function.

DIETRICH M, TRABER MG, JACQUES PF, CROSS CE, HU Y, and BLOCK G. Does gamma-tocopherol play a role in the primary prevention of heart disease and cancer? A review. *J. Am. Coll. Nutr.* 25:292-299, 2006.

The vitamin E family consists of eight isomers, including gamma-tocopherol and alpha-tocopherol. The American diet provides mainly gamma-tocopherol from soybean and canola oils, which has been shown *in vitro* and *in vivo* to possess anti-inflammatory and antioxidant properties. The authors reviewed epidemiological studies to determine whether gamma-tocopherol provides protection against heart disease or cancer. They found no evidence for a protective effect of gamma-tocopherol against heart disease and stroke, except in post-menopausal women. In men, gamma-tocopherol intake was associated with decreased risk of prostate cancer. Despite the rapid clearance of gamma-tocopherol from the body, its anti-inflammatory properties remain of great interest.

TRABER MG. Heart disease and single-vitamin supplementation. *Am. J. Clin. Nutr.* 85:293S-299S, 2007.

Vitamin E is a powerful fat-soluble antioxidant and anti-inflammatory agent. It becomes oxidized when it acts as an antioxidant but can be regenerated by vitamin C. Since oxidative stress is believed to play a role in the development of heart disease, the potential protective effect of vitamin E has been extensively studied. Results of intervention studies in patients with heart disease have been conflicting. Those results are difficult to interpret because actual levels of oxidative stress were not usually measured, nor were plasma levels of vitamin E. We know that vitamin E decreases biomarkers of oxidative stress and needs adequate vitamin C for optimal function, but we still lack precise knowledge about optimal intakes. In one large-scale, long-term primary prevention trial in healthy women, the daily intake of 600 IU of vitamin E was found to decrease mortality from heart disease by 24% and by 49% in women over 65 years old.

MCMACKIN CJ, WIDLANSKY ME, HAMBURG NM, HUANG AL, WELLER S, HOLBROOK M, GOKCE N, HAGEN TM, KEANEY JF JR, and VITA JA. Effect of combined treatment with alpha-lipoic acid and acetyl-L-carnitine on vascular function and blood pressure in patients with coronary artery disease. *J. Clin. Hypertens.* 9:249-255, 2007.

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Oxygen Club of California 2008 World Congress

The Annual Meeting of the Oxygen Club of California on *Oxidants and Antioxidants in Biology*, co-sponsored by the Linus Pauling Institute, will be held on March 12-15, 2008, at Fess Parker's Doubletree Resort in Santa Barbara, California. The meeting will feature keynote addresses by William Partridge and Bert Sakmann, as well as workshops on *Micronutrients and Brain Health* and *Alpha-Lipoic Acid and Thiol Redox Systems*. These workshops will consist of sessions on:

- Age-related metabolic pathways, mitochondrial nutrients, and neurodegeneration
- Flavonoids, polyphenols, cell signaling, and neuronal function
- Choline, amino acids, metals, and lipophilic micronutrients in brain health and function
- Oxidative stress and thiol redox circuits in cell function
- Alpha-lipoic acid in cell signaling and transcription
- Clinical aspects of alpha-lipoic acid

Twenty-nine scientists are scheduled to give presentations. Additionally, scientific posters will be presented on Thursday and Friday, and both young and established investigators will be recognized and honored with awards. For more information, please visit www.oxyclubcalifornia.org.



Based on evidence that mitochondrial dysfunction may contribute to the development of heart disease and recognizing that alpha-lipoic acid and acetyl-L-carnitine improve mitochondrial function and reduce oxidant stress, the investigators conducted a small, short-term clinical trial with patients with coronary artery disease. Patients were supplemented with 200 mg of alpha-lipoic acid and 500 mg of acetyl-L-carnitine twice daily for eight weeks. Such treatment resulted in a 2% increase in brachial artery diameter, a non-statistically significant decrease in blood pressure, and a significant decrease in systolic blood pressure in patients with initially elevated systolic blood pressure or with metabolic syndrome.

BLUMBERG JB and FREI B. Why clinical trials of vitamin E and cardiovascular disease may be fatally flawed. Commentary on "The Relationship Between Dose of Vitamin E and Suppression of Oxidative Stress in Humans". *Free Radic. Biol. Med.* 43:1374-1376, 2007.

Oxidative stress has long been implicated in the development of heart disease, but results of clinical studies designed to test the effectiveness of supplemental vitamin E in preventing heart attacks, stroke, or death from heart disease in patients with heart disease have been equivocal. Many epidemiological studies have reported associations between supplemental vitamin E intake and decreased incidence of heart disease, and *in vitro* and animal studies have identified several putative mechanisms of action, including antioxidant function, inhibition of smooth muscle cell proliferation, and prevention of platelet adhesion and aggregation. Conflicting results of clinical trials may be due to poor bioavailability—vitamin E must be taken with fat-containing food for absorption—or inappropriate duration or dose. In this commentary, the authors note that a recent study demonstrated that doses of vitamin E required to ameliorate oxidative stress in hypercholesterolemic patients, as measured by markers of lipid oxidation called F₂ isoprostanes, are four to eight times greater (1,600-3,200 IU) than doses commonly used in clinical trials. Hence, clinical trials may have failed simply because the vitamin E doses were inadequate to significantly lower oxidative stress. Levels of plasma vitamin E and biomarkers of oxidative stress were typically not measured in clinical trials.

Antioxidants

TRABER MG. How much vitamin E? ... Just enough! *Am. J. Clin. Nutr.* 84:959-960, 2006.

In this editorial, the author comments on a recent study (ATBC) showing that men with the highest serum concentration of vitamin E (alpha-tocopherol) had decreased risk of mortality from heart disease and cancer. The RDA for vitamin E (15 mg or 22.5 IU per day for adult men and women), set by the Food and Nutrition Board, is based on *in vitro* lysis of red blood cells taken from vitamin E-depleted men, not on the prevention of chronic disease. The new study of over 29,000 men followed for 19 years adds to the evidence that vitamin E helps prevent chronic disease. Based on other studies, an intake of about 100 IU/day may be necessary to increase the serum concentration of vitamin E to the protective levels described in the ATBC study. It is estimated that over 90% of Americans consume less than the RDA of vitamin E.

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 F₂ isoprostanes. *J. Nutr. Biochem.* 18:509-518, 2006.

Over ten percent of Americans do not consume the RDA of zinc, an abundant trace element involved in many critical cellular functions. It has been proposed that zinc functions as an antioxidant *in vivo*, leading the authors to conduct experiments with rats to determine potential antioxidant functions of zinc. Rats fed a zinc-deficient diet exhibited elevated levels of plasma F₂ isoprostanes, which are markers of lipid oxidation, and decreased levels of plasma uric acid—an important endogenous antioxidant—and hepatic vitamin E. The zinc-deficient diet did not appreciably affect levels of plasma vitamins C or E but did increase cytochrome P450 activity, which is involved in the metabolism of arachidonic acid. Oxidized arachidonic acid generates F₂ isoprostanes. Hence, it appears that the observed *in vivo* antioxidant function of zinc is indirect and related to its influence on plasma levels of uric acid and hepatic vitamin E, as well as on cytochrome P450 activity.



LOTITO SB and FREI B. Consumption of flavonoid-rich foods and increased plasma antioxidant capacity in humans: cause, consequence, or epiphenomenon? *Free Rad. Biol. Med.* 41:1727-1746, 2006.

Flavonoids are polyphenolic compounds in fruit and vegetables that exhibit potent antioxidant properties *in vitro*, and many scientists have assumed that they function as potent antioxidants *in vivo* as well. The authors previously showed that apple consumption by human subjects increased the antioxidant capacity of their plasma, but the observed effect was not due to vitamin C or flavonoids in apples, but rather to increased levels of plasma uric acid influenced by fructose in apples. Flavonoids are poorly absorbed into the bloodstream, extensively metabolized, and quickly excreted after absorption. In this review article, the authors examined the antioxidant effect of flavonoids in fruit and vegetables. It is estimated that the total daily intake of flavonoids is about 1,000 mg, with apples contributing over 20% of the flavonoid intake from fruit. One serving of apples, blueberries, red wine, or dark chocolate provides about 400 mg, 200-400 mg, 200-500 mg, or about 340 mg of flavonoids, respectively. Coffee and tea are also good sources of flavonoids, providing about 150-180 mg and 150-250 mg per serving, respectively. The authors note that it is possible that flavonoids may accumulate in certain tissues where they might act as antioxidants but that their health effects are more likely related to cell signaling, gene regulation, and other biological functions.

MUSTACICH DJ, LEONARD SW, DEVEREAUX MW, SOKOL RJ, and TRABER MG. alpha-Tocopherol regulation of hepatic cytochrome P450s and ABC transporters in rats. *Free Radic. Biol. Med.* 41:1069-1078, 2006.

The authors studied the metabolism and other effects of vitamin E (alpha-tocopherol) injected subcutaneously in rats. Daily injections elevated hepatic vitamin E levels, which then

began to decline (although remaining higher than baseline levels), suggesting that excess vitamin E was being eliminated by increased metabolism. This was confirmed by measurement of vitamin E metabolites, which increased over time. Certain cytochrome P450 enzymes involved in liver metabolism were found to be elevated in response to vitamin E injections. These same enzymes are critically involved in the metabolism of xenobiotics, including drugs. Therefore, the authors hypothesize that pharmacological intakes of vitamin E in humans may induce the P450 detoxification enzymes, consequently affecting the efficacy of pharmaceutical drugs.

Neurodegenerative Disease

PEHAR M, VARGAS MR, ROBINSON KM, CASSINA P, ENGLAND P, BECKMAN JS, ALZARI PM, and BARBEITO L. Peroxynitrite transforms nerve growth factor into an apoptotic factor for motor neurons. *Free Radic. Biol. Med.* 41:1632-1644, 2006.

Nerve growth factor (NGF) plays a critical role in neuronal development and maintenance. The investigators found that when NGF is exposed to peroxynitrite—an oxidant formed when superoxide reacts with nitric oxide—at physiologically relevant conditions, NGF is chemically modified and induces apoptosis (programmed cell death) of motor neurons *in vitro*. The authors further discovered that peroxynitrite caused oxidative damage to tyrosine, an amino acid in protein present in NGF, which was the mechanism for motor neuron apoptosis initiated by altered NGF. Urate, a physiological antioxidant present in blood, prevented the damage to tyrosine by peroxynitrite.

YE Y, QUIJANO C, ROBINSON KM, RICART KC, STRAYER AL, SAHAWNEH MA, SHACKA JJ, KIRK M, BARNES S, ACCAVITTI-LOPER MA, RADI R, BECKMAN JS, and ESTEVEZ AG. Prevention of peroxynitrite-induced apoptosis of motor neurons and PC12 cells by tyrosine-containing peptides. *J. Biol. Chem.* 282:6324-6337, 2007.

Peroxynitrite, formed from the reaction between nitric oxide and superoxide, is a strong oxidant that can damage biomolecules. It has been implicated in the development of inflammatory conditions and can damage cells by interfering with mitochondrial function and by damaging DNA and proteins through a process called nitration. In this paper, the authors report that peroxynitrite-derived radicals modify tyrosine residues in proteins, leading to the death of motor neurons *in vitro*. Tyrosine-containing peptides added to the culture medium scavenged peroxynitrite-derived radicals and protected motor neurons from death. These results offer new possibilities in neutralizing the damaging effects of peroxynitrite.

Aging

JUDGE S, JANG YM, SMITH A, HAGEN T, and LEEUWENBURGH C. Age-associated increases in oxidative stress and antioxidant enzyme activities in cardiac inter-fibrillar mitochondria: implications for the mitochondrial theory of aging. *FASEB J.* 19:419-421, 2005.

Mitochondria are intracellular organelles in which fatty acids are oxidized to produce chemical energy. There are two types of mitochondria in heart muscle cells (myocytes): subsarcolemmal (SSM) and interfibrillar (IFM). The authors investigated age-related changes in antioxidant status and

oxidative stress in cardiac SSM and IFM in young and old rats. Many markers of oxidative stress were elevated in IFM with age, whereas only one such marker,

protein carbonyls, was elevated in SSM with age. Antioxidant enzyme levels in both SSM and IFM increased with age, although catalase activity declined in SSM. Catalase is an antioxidant enzyme that scavenges hydrogen peroxide. Thus, hydrogen peroxide levels increased in SSM with age due to diminished catalase activity. Levels of glutathione, another endogenous antioxidant, were lower in IFM regardless of age. Since cardiac myocytes are postmitotic cells (i.e., incapable of cell division) and have a high demand for oxygen, they may be especially vulnerable to oxidative stress and damage. When mitochondrial dysfunction implicated in the age-related decline of cardiac function is examined, special attention should be given to the different characteristics of the two populations of mitochondria in cardiac myocytes. Conflicting results of previous studies may be due to isolation procedures that do not effectively separate SSM and IFM.

SMITH AR, VISIOLI F, FREI B, and HAGEN TM.

Age-related changes in endothelial nitric oxide synthase phosphorylation and nitric oxide dependent vasodilation: evidence for a novel mechanism involving sphingomyelinase and ceramide-activated phosphatase 2A. *Aging Cell* 5:391-400, 2006.

The authors examined the age-related decline in nitric oxide activity in rats. Nitric oxide is a signaling molecule critically involved in the function of smooth muscle cells that line blood vessels. Impairment of nitric oxide activity leads to increased rigidity of vessels, which is a hallmark of aging and heart disease. The authors found that age-related post-translational modifications (phosphorylation) of proteins involved in nitric oxide synthesis partly explain this phenomenon. They found that ceramides—lipids composed of sphingosine and fatty acids that are found in cell membranes—accumulate with age, resulting in disruptions in cell signaling that interfere with nitric oxide synthesis. A compound that inhibits the abnormal increase in ceramides was found to improve endothelial function and relaxation in aged aortas. The authors speculated that age-related declines in glutathione—an endogenous antioxidant—may be responsible for the increased ceramide activity in aged endothelium.

VISIOLI F and HAGEN TM. Nutritional strategies for healthy cardiovascular aging: Focus on micronutrients. *Pharmacol. Res.* 55:199-206, 2007.

The authors note that age is the major risk factor for heart disease and that over 40% of elderly people in developed countries have inadequate nutrition. Socioeconomic and lifestyle factors, as well as age-related physiological changes, play a role in this malnutrition. For example, atrophic gastritis inhibits the absorption of vitamin B12, which can lead to hematological diseases and neuropsychiatric disorders, and



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iatrogenic illness caused by the use of multiple medications can also impair nutrient intake. B vitamins lower homocysteine levels, improve cell function in blood vessels, and exert anti-inflammatory effects. With age, oxidative stress increases as the production of oxidants increases and as stress response enzymes and other antioxidants diminish. One consequence of these phenomena is the deterioration of cardiac function. Many studies have found that the consumption of fatty fish or the use of omega-3 fatty acid supplements helps protect against heart disease and sudden death from cardiac failure, possibly through anti-arrhythmic and anti-inflammatory activity or by stabilization of atherosclerotic plaque. Vitamin E may be helpful in preventing heart disease, but studies have reported conflicting results. Low vitamin C intake in the elderly is associated with higher mortality from stroke, and high vitamin C intake lowers mortality from heart disease. A meta-analysis concluded that the elderly need more vitamin C to attain plasma levels achieved in younger adults with much lower intakes. This may be due to age-related changes in one of the cellular uptake mechanisms for vitamin C. In clinical trials, vitamin C has been shown to lower blood pressure and to improve vasodilation. Additionally, dietary flavonoids from fruit and vegetables may help attenuate inflammation and inhibit platelet aggregation.

Nutrition and Other Topics

POPKIN BM, ARMSTRONG LE, BRAY GM, CABALLERO B, FREI B, and WILLETT WC. A new proposed guidance system for beverage consumption in the United States. *Am. J. Clin. Nutr.* 83:529-542, 2006.

We are familiar with the food pyramid established by the U.S. Department of Agriculture to encourage better dietary habits. In this paper, the authors propose a “beverage guidance system” to help us choose healthful beverages. Presently, caloric intake from beverages represents about 20% of total energy intake for most Americans and contributes to the obesity trend among children and adults. Calorically sweetened beverages are mainly responsible for an increase in caloric intake, and the proposed guidelines rank beverages in value based on caloric and nutrient content and on health benefits and risks. The authors ranked beverages in the following order based on those parameters: water, tea and coffee, low-fat and skim milk and soy beverages, noncalorically sweetened drinks, beverages with some health benefits (fruit and vegetable juices, whole milk, alcoholic drinks, and sports drinks), and, lastly, calorically sweetened beverages, which should be avoided. Although it is not possible to recommend specific amounts of these beverages, it may be possible to reduce caloric intake from beverages to about 15% of daily total energy intake by following the guidelines. The authors suggest drinking 20-50 fluid ounces of water each day, which accounts for about 20-50% of the recommended total daily fluid intake of about 100 fluid ounces.

HIGDON JV and FREI B. Coffee and health: a review of recent human research. *Crit. Rev. Food Sci. Nutr.* 46:101-123, 2006.

Coffee contains over one thousand chemical compounds, including caffeine, chlorogenic acid, and diterpenes (cafestol and kahweol). Caffeine content in brewed coffee ranges from

72-130 mg per eight-ounce (about 240 ml) serving but can be as high as 282 mg per eight-ounce serving, depending on variations in roasting, grinding, type of bean, brewing time, etc. Chlorogenic acid is a polyphenol that is converted to caffeic acid and quinic acid in the gut. These acids may act as physiological antioxidants. A number of studies have found that diterpenes in boiled coffee raise serum total and LDL cholesterol levels. Diterpenes are removed by paper filters during brewing. Coffee also contains magnesium and potassium and is a significant source of niacin. Epidemiological studies found that coffee may help protect against type 2 diabetes, Parkinson's disease, colorectal and liver cancer, and cirrhosis. On the other hand, high coffee consumption has been associated in some epidemiological studies with increased risk of heart disease, while other studies have not found that association, despite the observations that coffee consumption increases plasma homocysteine levels and raises blood pressure in non-hypertensive subjects and that boiled coffee raises cholesterol levels. Coffee may also impair iron and zinc absorption and increase the risk of osteoporotic fractures in older adults. The maternal intake of more than three cups per day may increase the risk of spontaneous abortion and impair fetal growth.

PACHER P, BECKMAN JS, and LIAUDET L. Nitric oxide and peroxynitrite in health and disease. *Physiol. Rev.* 87: 315-424, 2007.

In this lengthy review, the authors provide comprehensive information on the research history of nitric oxide and peroxynitrite and their roles in health and disease. Nitric oxide is the first gas found to have a role as a physiological signaling molecule. Over 25 years ago investigators found that an uncharacterized molecule called endothelium-derived relaxing factor (EDRF) played an important role in relaxing smooth muscle cells around blood vessels, permitting dilation and normal function. Years later, EDRF was identified as nitric oxide, and a number of researchers shared the Nobel Prize for identifying its cell-signaling properties. Nitric oxide is synthesized in the body from the amino acid arginine by nitric oxide synthases. It diffuses through tissues and is converted to nitrate in red blood cells. Nitric oxide can combine with the superoxide radical to form peroxynitrite, which is a very powerful oxidant used by immune cells to destroy pathogens. However, excess peroxynitrite can damage biomolecules like proteins, lipids, and DNA, as well as mitochondria, often resulting in cell death. The body synthesizes an antioxidant enzyme called superoxide dismutase to convert superoxide to hydrogen peroxide and stable oxygen so that production of excess peroxynitrite is partly mitigated. Peroxynitrite itself decomposes into radicals capable of damaging biomolecules. Peroxynitrite has been implicated in cardiovascular dysfunction and pathology, including heart failure, atherosclerosis, and hypertension; inflammatory diseases like arthritis and inflammatory bowel disease; diabetes; stroke and reperfusion injury; cancer; and neurodegenerative diseases like multiple sclerosis, Parkinson's disease, Alzheimer's disease, and ALS. Antioxidants like urate, vitamins C and E, and polyphenolic compounds found in fruit and vegetables may help protect against peroxynitrite damage. Urate is an endogenous antioxidant formed in the body from DNA breakdown products called purines, which are also found in foods like sardines and organ meat.

ZHANG W-J, WEI H, HAGEN T, and FREI B. alpha-Lipoic acid attenuates LPS-induced inflammatory responses

by activating the phosphoinositide 3-kinase/Akt signaling pathway. *Proc. Natl. Acad. Sci. USA* 104:4077-4082, 2007.

Monocytes are immune system cells that circulate in the blood. When they migrate into tissues, they become macrophages and engulf pathogens. When macrophages ingest oxidized cholesterol in the arterial wall, they become foam cells and contribute to the development of atherosclerotic lesions. When monocytes are activated by bacterial toxins, they elicit a pro-inflammatory response that can lead to sepsis (septic

shock) and its sequelae, including organ failure and death. The authors exposed monocytes *in vitro* to a bacterial toxin and found that alpha-lipoic acid added to the culture medium inhibited the acute inflammatory response by activating a specific biochemical signaling pathway (PI3K/Akt). The effect of alpha-lipoic acid on this pathway was confirmed *in vivo* using mice. The authors also found that mice injected with alpha-lipoic acid prior to exposure to the bacterial toxin were significantly protected from death. **LPI**

PUBLICATIONS LIST

2004 (addendum)

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Developments

Scott Palmer
LPI Director of Development

For the Linus Pauling Institute, the past 18 months have been full of anniversaries and significant milestones. In 2006 we celebrated both the tenth anniversary of the Institute's move from Palo Alto, California, to Oregon State University and the twentieth anniversary of the publication of Dr. Pauling's international best seller, *How to Live Longer and Feel Better*. OSU Press reprinted the book last year, and the first printing has already sold out. A second printing is in the works.

This year marks Dr. Balz Frei's tenth anniversary as Director of the Institute, and in March of 2008 Dr. George Bailey, one of our original Principal Investigators and a Distinguished Professor at Oregon State University, will officially retire, concluding a remarkable scientific career in cancer chemoprevention. However, the construction of the Linus Pauling Science Center may be the most significant event in the history of the Linus Pauling Institute.

On October 26th, we celebrated the public announcement of OSU's first ever Capital Campaign and the centerpiece of the Campaign: the Linus Pauling Science Center. Raising money for the Center was a top priority for the \$620 million University-wide Capital Campaign.

While many people have been involved with the effort to raise the funds for this project, it was the leadership and advocacy of Dr. Edward Ray, President of Oregon State University, coupled with the vision and generosity of the Wayne and Gladys Valley Foundation and Al and Pat Reser, along with a handful of other donors, that made this building possible.

As Dr. Frei noted in his column, now that we have secured the funding for the building, the Linus Pauling Institute is getting ready to launch a major new research initiative in healthy aging. The goal of this initiative is to help everyone achieve their maximum healthspan through optimum health—we want people to live better, not just longer. We know that micronutrients, diet, and lifestyle play a significant role in helping people age more gracefully, and through this new research initiative we want to find out exactly why.

Many different research organizations have a new-found focus on aging. Often that focus is disease specific; that is, the goal is to try and find a cure for an existing health condition associated with aging once it has manifested itself. LPI has the opportunity to do something different—further our knowledge of how and why we age at the cellular and molecular level to better understand how we can prevent the increased susceptibility to disease as we age.

However, to do that we need to build our program by recruiting new faculty and equipping new labs with modern equipment—all of which takes money. Our future success with this initiative will continue to rely heavily on

our many donors and friends who have supported us in the past. For those that are interested in supporting our Healthy Aging Program, there will be many opportunities to participate, including:

- Endowed chairs in healthy aging research
- Professorships in healthy aging and neurodegenerative diseases
- Fellowships to support graduate students
- Gifts to purchase new equipment
- Program support for research projects

Because the funding for the building itself is already in place, we have a unique opportunity to give special recognition to donors who support our Healthy Aging Program or any other research projects in LPI through a gift of \$10,000 or more. Between now and the grand opening of the Center in 2010, individuals or corporations at that level will be recognized on a plaque in the new building. Donors who give \$25,000 to LPI will have the opportunity to name a room in the new Center. Naming opportunities for rooms or facilities range from \$25,000 to over \$1,000,000.

Gifts can be in the form of an outright gift of cash, a pledge payable over a period of several years, gifts of stock or real estate, a Charitable Gift Annuity or Charitable Remainder Trust or, under certain circumstances, simply including LPI in your will. The bottom line is that your gift will make a significant difference.

For our donors and supporters who will be 70½ years or older this year, you can still make a gift to LPI from your pension plan. The Pension Protection Act of 2006 allows individuals aged 70½ or older to make gifts up to \$100,000 to qualified charitable organizations like ours using funds transferred directly from their IRAs. An IRA gift of \$25,000 or more qualifies as a naming opportunity for the Linus Pauling Science Center.

Someone who does give money from their IRA does not have to pay taxes on the amounts transferred. There are other benefits to giving from your IRA. However, this law sunsets on December 31, 2007, so if you are interested in exploring this option please let us know soon!

For more information on how to give to the Linus Pauling Institute from your IRA, how to include the Institute in your will or estate plan, or to find out more about naming opportunities in the Linus Pauling Science Center, please contact me at (541) 231-6751 or by email: scott.palmer@oregonstate.edu. **LPI**

LPI is grateful for the bequests we have received from the following friends this past year:

Nancy Bradford	John Holterhoff
Evelyn Bullock	David Holtzman
Matilda Charles	Arthur Kahn
Ethel Golder	Virginia Martindale
	Karla Pepe



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Gifts in support of research efforts can be made at any time. Checks should be payable to
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George Bailey Retires

Dr. George Bailey, LPI Principal Investigator and Distinguished Professor Emeritus of Environmental and Molecular Toxicology, has announced that he will retire in March 2008. Dr. Bailey earned his Ph.D. in Biochemistry at the University of California-Berkeley and has served as a Professor at OSU since 1979. He has written over 150 research papers and has been awarded funding from the National Cancer Institute and National Institute of Environmental Health Sciences for many years. He has been recognized with many awards, including the Sixth Prince Hitachi Prize in Comparative Oncology. Dr. Bailey formerly directed OSU's Marine/Freshwater Biomedical Sciences Center, where he developed trout as an experimental model for human carcinogenesis and cancer chemoprotection studies. Those studies led to a collaborative project in China, where people chronically exposed to the dietary carcinogen aflatoxin B1 were supplemented with chlorophyllin, a derivative of chlorophyll. Chlorophyllin supplementation reduced the amount of cancer biomarkers in blood and urine, suggesting that chlorophyllin binds to aflatoxin and helps protect against liver cancer. Dr. Bailey has been a valued member of LPI, and we wish him continued success in his retirement.

Look for these informative articles inside!

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Micronutrient Research for Optimum Health

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