

The Linus Pauling Institute

FALL/WINTER 2014 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

As many of you know, our former Director of Development, Michele Erickson, left LPI earlier this year to become the Director of Development for OSU's College of Business. We are happy for her promotion and wish her success in her new position. I am very pleased to announce that LPI has a new Director of Development, Marlys Amundson, who was with OSU's College of Engineering for many years. Marlys has a lot of passion for LPI's vision and mission and will work hard to raise funds to help keep our research and education enterprise in good shape. As she discusses in her column on page 15, federal funding for biomedical research has been stagnant for many years, except for a brief stimulus period during the recent recession. This has had the unfortunate consequence of inhibiting bright, young scientists from entering academia, where the expectations for constant grant funding are high. It has also interrupted the research momentum of scientists who lose grant funding. Many scientists compete for extremely limited funding, and the probability of being awarded grants, even for creative and important work, has significantly declined. Marlys is excited to be working with you and LPI to help us advance our research into achieving long and healthy lives for everybody.



Marlys Amundson

To help increase our chances for successful grant applications, Dr. Joe Beckman, one of LPI's principal investigators, has agreed to take on the new position of Associate Director for Research at LPI. Many of you are familiar with Joe's exciting and innovative research on Lou Gehrig's disease, also known as amyotrophic lateral sclerosis, or ALS. This research has been summarized in previous newsletters, and you can read about Joe's latest

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New Benefits of Vitamin E

An Interview with
Debbie J. Mustacich, Ph.D.
Associate Professor (Senior Research)

Q. Are you from the Pacific Northwest?

A. Yes, I am. I grew up mainly in Gig Harbor, Washington. We lived on an island just outside the city.

Q. You earned your Ph.D. in biochemistry and biophysics from Oregon State University (OSU). What was your thesis about?

A. My thesis research was about the role of microtubules and multi-drug resistant transporters in the secretion of alpha-tocopherol—vitamin E—in bile and then reuptake in the gut.

Q. In 2004, you moved from the Arizona Cancer Center at The University of Arizona to join the Linus Pauling Institute (LPI) as a research associate in Maret Traber's lab. What motivated you to come to LPI?

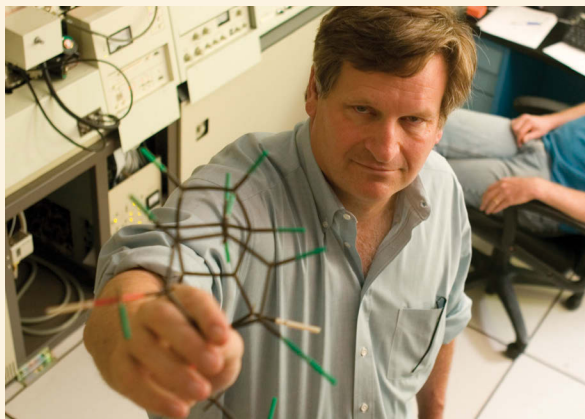
A. I was familiar with several of the LPI investigators through scientific meetings, and I knew about the collegial atmosphere here. That's what I was looking for, and that's why I came here. I left a very high-paying industry position to start over again in academia.

Q. Your academic appointment here at OSU is in the Department of Biomedical Sciences in the College of Veterinary Medicine. Does that stimulate collaborations between that college and LPI?

A. Yes, it does. There are several collaborations between the vet school and myself, and other LPI investigators have had collaborations, too. It's part of the reason I was invited to become part of the College of Veterinary Medicine—in hopes to have more connections between them and LPI.

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Continued from cover — From the Director



Dr. Joe Beckman

work in this area on page 10 of this newsletter. As Associate Director for Research, Joe will help me to coordinate LPI's research program and assist our principal investigators with individual and multi-investigator grant applications. Joe will work to integrate and expand our core laboratories in order to develop screening assays for dietary factors that promote healthspan. He will also help to advance our metabolomics efforts that will lead to a better understanding of the roles of metabolites in health, coordinate the acquisition and use of instruments, and, importantly, help LPI faculty carry out studies with human subjects. Joe will assist with research contracts and work to increase private support for LPI. I am very pleased to have Joe's expert help and guidance in managing our research program.

In my last two columns, I outlined our recently concluded strategic planning process and the new goals and key initiatives that emerged, including 1) establishing LPI's leadership in advancing healthspan through innovative research, 2) diversifying and increasing funding for research and public education, 3) effectively communicating our message, and 4) improving public health through community engagement. We are in the initial phase of implementing several of these objectives and have been gaining some momentum and developing a lot of enthusiasm. I will keep you informed of our progress through periodic emails and this column. I'm glad that you are joining us on this adventure!

LPI



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Continued from cover — New Benefits of Vitamin E

Q. Do a lot of the veterinary students get involved in research?

A. Yes. The vet school has a program through the Department of Biomedical Sciences. I usually have one or two veterinary students every summer. They provide fellowships for approximately 10 students every summer to work in the various labs of Biomedical Sciences faculty.

Q. At The University of Arizona, you worked on a protein called thioredoxin. What important cellular functions does thioredoxin have?

A. Thioredoxin has several functions. Thioredoxin protects thiols—sulfur-containing compounds—within the cell. It is part of the thioredoxin system and is involved in antioxidant defense. We were interested in cancer, so we were looking at its role in cell signaling. Thioredoxin is important in maintaining the reduced state of certain cysteines within signaling proteins that induce cells to proliferate. We found that when thioredoxin is over-expressed in human cells, there is increased cell growth and resistance to apoptosis, or programmed cell death.

Q. How is thioredoxin implicated in cancer?

A. Thioredoxin is expressed at high levels in a number of human tumors, including lung, pancreas, colon, gastric, and breast cancer. So that was the interest of our lab—does thioredoxin play a role in carcinogenesis and should we target thioredoxin for cancer prevention and/or treatment?

Q. How does thioredoxin promote cancer?

A. Although the presence of high levels of thioredoxin in tumors has been linked to increased tumor proliferation and decreased patient survival, we demonstrated that increased expression of thioredoxin by itself does not promote cancer.

Q. Also in Tucson, you showed that transgenic mice expressing increased levels of thioredoxin in their skin cells developed more tumors when exposed to a topical carcinogen. How variable are thioredoxin levels in human skin cells, and is there the same correlation in humans with increased risk for tumorigenesis?

A. Thioredoxin is expressed at low levels in non-sun-exposed human skin within hair follicles and sebaceous glands but is not generally seen in the epidermis. However, UVA and UVB exposure increases thioredoxin in skin cells, and thioredoxin is significantly elevated in sun-damaged human skin. In our mouse studies, mice expressing elevated thioredoxin did not develop tumors unless they were treated with both a carcinogen and promoting agent. All of the mice treated with the carcinogen and promoter developed tumors, but those with elevated skin thioredoxin developed more tumors, and the tumors tended to be more aggressive.

Q. UV radiation increases thioredoxin levels in human skin, presumably to protect against DNA damage. But could the increased levels also contribute to cancer development?

A. Initially, the elevated levels would protect the cells from damage and help them to repair themselves. Nevertheless, some cells may get repeated DNA damage from the sun, and the elevated thioredoxin could protect a damaged cell that has not been repaired from undergoing apoptosis. Thus, in normal cells, thioredoxin will be protective. Elevated thioredoxin by itself isn't going to lead to cancer.

Q. Can thioredoxin levels be affected by diet or drugs?

A. There are some drugs that may increase thioredoxin, but I'm not aware of dietary agents that affect it.

Q. When you joined Maret Traber's lab in LPI, you worked on vitamin E. One of your early studies showed that rats metabolize excess vitamin E so that it doesn't accumulate. Do you know how that's accomplished? Why is that important?

A. It's important because most lipid-soluble vitamins can accumulate in your tissues, unlike the water-soluble vitamins that tend not to accumulate and are readily excreted in urine. When we injected rats with extremely high levels of alpha-tocopherol—vitamin E—we found that metabolism and excretion of alpha-tocopherol increased to eliminate the excess. This included increases in some of the cytochrome P450 enzymes present in the liver that are known to metabolize foreign compounds.

Q. Is that a case where the xenobiotic-metabolizing enzymes recognize the very high level of vitamin E and then metabolize it as if it were a foreign substance?

A. Yes. Vitamin E, like any vitamin, is not made in the body. Technically, it is a xenobiotic—it's a foreign chemical as far as the body is concerned. If the levels get too high, then mechanisms for metabolism and excretion may be induced to rid the body of the excess and prevent possible toxic effects. This is the case for excess vitamin E.

Q. You were injecting vitamin E into the rats. Would you expect the same thing to occur when very large amounts of vitamin E are taken orally?

A. When vitamin E is taken orally the amount that may be absorbed in the gut is low, so you would not be able to reach the liver concentrations that we saw with our injection protocol. There was one mouse study done prior to my arrival at the Linus Pauling Institute in which rats were fed very high levels of vitamin E in the diet for

several months. That resulted in slight elevations of one of the P450 enzymes that we saw induced with injections. To date, we haven't seen evidence of drug interactions due to altered P450s following oral supplementation with vitamin E.

Q. More recently you worked on spinal cord compression injuries in rats, showing that an intravenous infusion of antioxidants and anti-inflammatory compounds after the injury improved recovery of neurologic function, decreased inflammation, increased vascularization, and increased survival. What was infused?

A. This was a collaborative project with a veterinary orthopedic surgeon, Dr. Wendy Baltzer, and was set up to closely replicate a therapeutic treatment relevant to both human and veterinary injuries. We used commercially available lipid emulsions. One emulsion contained mainly long-chain fatty acids, the second contained a mixture of long-chain and medium-chain fatty acids, and the third contained long-chain, medium-chain, and omega-3 fatty acids—fish oil—as well as elevated levels of vitamin E.

Q. Which was the most effective emulsion?

A. The emulsion containing medium-chain and omega-3 fatty acids with elevated vitamin E was the most effective. We actually had a few rats that were able to walk normally three weeks following their injury.

Q. What explains those results?

A. We know that biochemical damage continues to occur after the initial mechanical injury. This is referred to as secondary spinal cord injury. The mechanism for this is undetermined, but we hypothesized that oxidative stress, specifically lipid peroxidation, was playing a role in this secondary injury. That's why we chose to use vitamin E in the treatments. Inflammatory responses are also implicated in secondary injury, so we included fish oil, which is anti-inflammatory. The emulsion containing high levels of vitamin E and omega-3s helped decrease indices of both lipid peroxidation and inflammation and significantly improved the recovery of our rats.

Q. Do you think that much more work is needed before that could be used to treat people with compression spinal cord injuries?

A. I think that there's more to be done to further improve recoveries. That said, these emulsions are commercially available in Europe and are currently being used to improve the recovery of surgical patients.



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Q. Not spinal injuries?

A. No, they have not yet been used with spinal injuries to my knowledge. They have been shown to improve the speed of recovery—reducing time in the hospital—for surgical patients.

Q. That's very interesting because Nathens at the University of Washington published a paper 12 years ago showing that surgical trauma patients who got 1,000 mg of intravenous vitamin C and 1,000 IU of oral vitamin E fared much better than patients who did not get those antioxidants, with lower organ failure rates and fewer days in the ICU. It was a randomized controlled trial with about 600 patients.

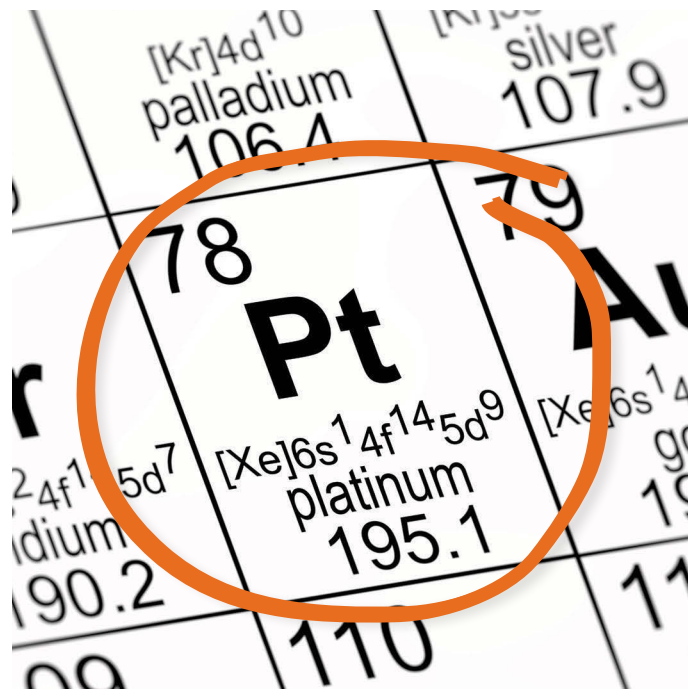
A. We see the same kind of data in small studies that have been done in Europe. Another reason we thought that vitamin E would be beneficial is because injured rodents that were supplemented with vitamin E prior to experimental injury showed improved recovery compared to non-supplemented rodents. However, since we don't know when we are going to get a spinal cord injury, we did our study as a post-injury treatment. In our pilot study, we merely gave a 24-hour intravenous treatment following the injury. We did not give any oral supplementation after that. I suspect that if we repeated the IV treatments at additional intervals after the injury and/or gave oral supplements after the initial IV treatment, the rats' recoveries would be even better.

Q. Cisplatin is a platinum compound used to treat ovarian and lung cancer and lymphoma but has serious toxic side effects, including neurotoxicity and kidney damage. What do your studies with vitamin E show?

A. It's known that cisplatin decreases plasma vitamin E levels in cancer patients. We see similar decreases of plasma vitamin E in rats given cisplatin, but because we can look at all the tissues in the rats, we also see significant decreases in organs. Importantly, we see decreases of vitamin E in the various regions of the spinal cord and ganglia.

Q. Does that contribute to neuropathy?

A. The mechanism for cisplatin neuropathy is unknown. However, the clinical features of cisplatin-induced neuropathy are similar to those seen in vitamin E deficiency neuropathy. Nervous tissue contains lots of lipids. Vitamin E protects lipids from oxidation but in the process may be depleted from the tissues. We believe platinum catalyzes the process of lipid peroxidation within the nervous tissue, leading to depletion of vitamin E and that this depletion of vitamin E plays a central role in cisplatin-induced neuropathy. When we



give female tumor-bearing rats high doses of vitamin E the day before we give them cisplatin, we don't see decreases in the spinal cord vitamin E. In fact, we see increased levels in tissues, sometimes elevated above those in normal, healthy female rats.

Q. And there's no effect on the anticancer efficacy of cisplatin?

A. The great thing that we found in our study is that vitamin E actually improves the anti-tumor efficacy of cisplatin. We observed a significant decrease in the number of rats with tumors in rats that received cisplatin plus vitamin E compared to cisplatin alone. In fact, some of the rats receiving the combination had no evidence of tumors.

Q. Do you have any idea how vitamin E prevents the accumulation of platinum in normal tissues?

A. We don't know exactly why platinum accumulates in some tissues more than others. Even the molecular mechanism of how it kills cancer cells has not been fully determined, particularly its role in apoptosis. We know that it incorporates into DNA and forms adducts. The ability of a particular tissue to repair those DNA adducts and remove the platinum may determine whether a cell lives or dies. In our study we saw a decreased accumulation of platinum in the spinal cord, which is very important, but we don't yet know the mechanism. Equally important, vitamin E does not alter the accumulation of platinum in the ovarian tumors.

Q. Does the vitamin E combined with cisplatin result in decreased kidney damage?

A. That wasn't a focus of the study. We did collect the kidneys, and we didn't see histologic differences, although there was an improved ability to concentrate the urine in rats receiving vitamin E with the cisplatin compared to cisplatin alone. One of the signs of kidney damage in rats given cisplatin is an increased volume of very dilute urine.

Q. What other animal studies need to be done?

A. We would like to look at the combination of vitamin E and vitamin C plus cisplatin. We think that this combination would further improve the efficacy, as well as further decrease side effects from the unwanted damage in tissues outside the tumors. I don't believe that the effect of vitamin C on neuropathy has been investigated, and that would be very important as well.

Q. Can your results be extrapolated to other chemotherapeutic drugs?

A. They could be extrapolated to other chemotherapeutic drugs depending on the mechanism for the chemotherapeutic drug. Many chemotherapeutics cause their side effects through various mechanisms of oxidative stress, and some kill cancer cells by similar mechanisms. Other cancer drugs, including cisplatin, cause oxidative stress to both tumor and normal tissues but kill the cancer cells in a manner that does not require oxidative stress. Those are the chemotherapeutics that might be beneficially combined with antioxidants.

Q. What are you working on now?

A. We are continuing to work on the cisplatin studies. We need to further characterize the antioxidant levels and tissue damage in the rat spinal cords. In addition, we are examining the tumors histologically to look at markers of cell proliferation and apoptosis. Also, I am collaborating with veterinarians in the veterinary school on work with Lyprinol and osteoarthritis in dogs.

Q. What is Lyprinol?

A. It comes from the green-lipped mussel. It's a collection of several different lipids (free fatty acids) and has demonstrated anti-inflammatory properties. We measured F₂-isoprostane levels, which are markers of oxidative stress, specifically lipid peroxidation, and found they were significantly decreased in Lyprinol-supplemented dogs with osteoarthritis.

Q. Was there any change in behavior in the dogs?

A. The veterinarians are still processing those outcomes.

Q. Is Lyprinol injected or given orally?

A. It's taken orally.

Q. Caitlin Crimp, one of your undergraduate students in the Honors College at OSU, worked on the protection by vitamin E against polycyclic aromatic hydrocarbons—PAHs—that are ubiquitous environmental carcinogens caused by the incomplete combustion of carbon fuels. What did she find?

A. She found that when we gave high levels of vitamin E to our rats over a period of a few days with the purpose of inducing the P450s that we discussed earlier, we actually altered the metabolism of the PAH, benzopyrene. We pretreated the rats with vitamin E over a five- to seven-day period—sufficient to induce specific P450s—and then gave the rats a dose of benzopyrene. I hypothesized that the PAH would be quickly metabolized to non-toxic metabolites that would then be excreted. And that is exactly what we found. We saw a shift in the metabolism of the PAH from the toxic DNA-adducting metabolites to the non-toxic non-DNA-adducting metabolites and elevated excretion of both the parent compound and the metabolites in urine and bile.

Q. Was the dose of PAH given by injection much greater than you would be exposed to in the environment?

A. Yes, it was more than the average person would be exposed to environmentally. Our study used a high dose of PAH, the kind of occupational exposure to which workers like forest fire fighters, roofers, first responders, and road crews might be exposed. However, the mechanism should be relevant for lower doses or exposures. We would like to do further studies with lower, long-term doses to replicate additional exposure scenarios.

Q. Were there any gender differences in the response of the rats to the treatment?

A. Yes. That was very interesting because the female rats actually had a greater response. Other scientists conducted a study in humans who smoked. The smokers

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were given vitamin E orally, and the female smokers responded—they had fewer DNA adducts in their white blood cells than male smokers. Although the human findings didn't reach statistical significance due to the small sample size, we saw a similar trend in our rat study.

Q. Is your work funded primarily by grants?

A. Yes. Currently, the cisplatin work is funded by the American Cancer Society. The small veterinary studies, such as the Lyprinol studies, as well as the pilot study in spinal cords, were funded by the Linus Pauling Institute, the College of Veterinary Medicine, and the Division of Health Sciences. The NIH funded the vitamin E and PAH studies.

Q. How do you like working in the new Linus Pauling Science Center?

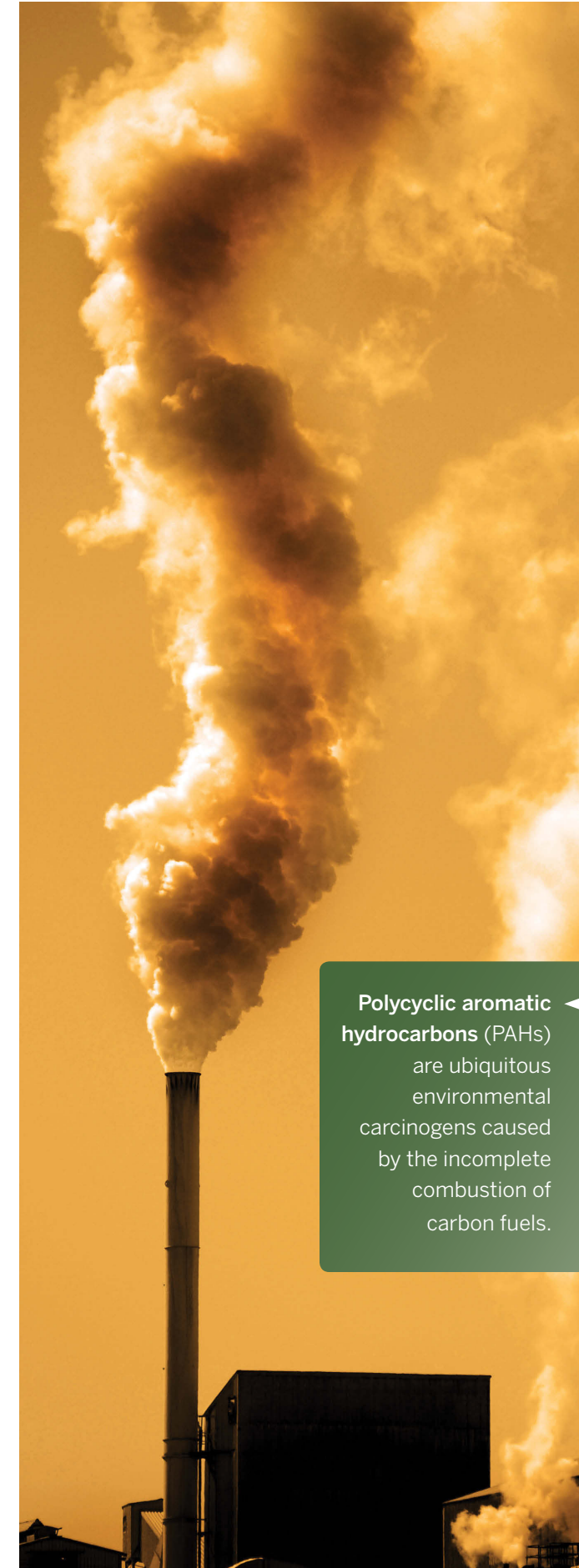
A. I love it. It's spectacular to have such a nice facility along with a nice group of researchers. We really like the open labs. I think they lead to increased collaborations. My students like it, and it's quite popular with the veterinary students to work in my lab.

Q. Are you able to attract a lot of undergraduate students or do you have some graduate students?

A. I do not have graduate students in my lab, but I have no shortage of undergraduates. I turn undergraduates away on a regular basis because I don't have room for them. I prefer to keep a small group of students so that I have time to work personally with each of them on their training. I generally have two veterinary students in the summer. It's good for me, and it's good for them because we do animal studies that allow them to get some hands-on experience giving the injections, handling the animals, learning about care issues, and applying scientific methods. They are very good at monitoring animal health because they already have been trained for that. And I get excellent undergraduates—many have received awards for their work. Most of my undergraduates and veterinary students have presented their work at international scientific meetings. They do great work, and I enjoy them.

Q. What do you like to do in your free time when you are not working in the lab?

A. My main activity is golfing, and I usually do an annual ski trip to visit my brother and family in Canada. We recently went to France to visit my sister and her family, but mostly I golf and visit my nieces and nephews whenever possible. **LPI**

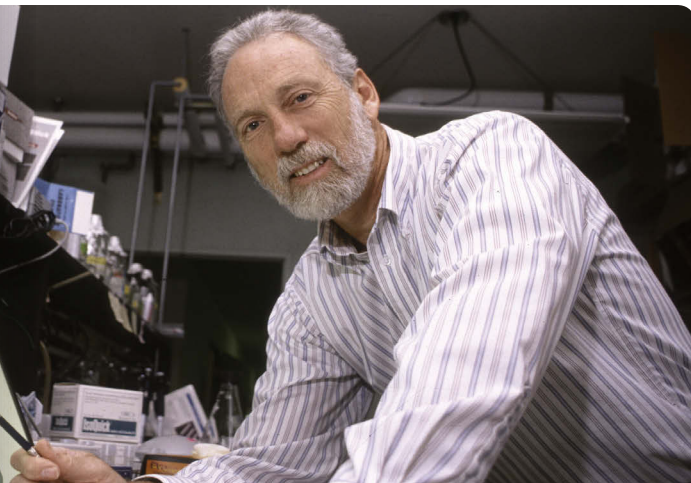


Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental carcinogens caused by the incomplete combustion of carbon fuels.

IN MEMORIAM:

Dr. George Bailey

1941-2014



Dr. George Bailey, OSU professor emeritus in environmental and molecular toxicology and former LPI principal investigator, died on October 20th after a serious illness. He retired in 2008 after a nearly 30-year career at Oregon State University and over five years as a principal investigator in LPI.

George earned a B.S. in chemistry at the University of Southern California, followed by a Ph.D. in biochemistry from the University of California-Berkeley. He joined OSU in 1979 as an assistant professor in the Department of Food Science and Technology and was honored in 1998 with the title of Distinguished Professor in the Department of Environmental and Molecular Toxicology. He served as the director of OSU's Marine and Freshwater Biomedical Sciences Center from 1985 to 2002. George published over 150 papers in peer-reviewed scientific journals and won several awards for outstanding research, including the prestigious Prince Hitachi Prize for Comparative Oncology in 2001. On November 12th, he was awarded posthumously the Discovery Award by the Medical Research Foundation of Oregon Health & Science University, an honor previously bestowed on LPI's Drs. Balz Frei and Joe Beckman. Over the years, he has also mentored many graduate students at OSU.

In 1995, George was one of several members of a faculty advisory committee established by Dr. Richard Scanlan, OSU's then-Dean of Research, to advise OSU's former President, Dr. John Byrne, on the possible establishment of the Linus Pauling Institute at OSU. Those deliberations were successful, and the Linus Pauling Institute moved from Palo Alto, California, in 1996 to become a research institute at OSU.

George was especially interested in how dietary phytochemicals (plant-derived chemicals that may affect health) inhibit cancer in animal models and the extrapolation of that research to humans. Using the rainbow trout model, George and his colleagues were able to achieve unprecedented statistical power using the dose-dose matrix experimental design in which various doses of carcinogens are tested against various doses of phytochemicals that are expected to protect against cancer. He also assembled the most

comprehensive database of carcinogen-DNA adducts as early biomarkers for cancer chemoprotection by phytochemicals in any animal model.

George and his colleagues showed that liver cancer in trout induced by aflatoxin, a carcinogen formed by mold that grows on damp nuts and grain, could be inhibited by chlorophyllin (a stable derivative of chlorophyll) through a blocking mechanism. Aflatoxin binds to chlorophyllin in the gut, and the complex is harmlessly excreted, preventing its absorption into the blood stream and delivery to the liver. George and his colleagues conducted a human trial in China where people are unavoidably exposed to dietary aflatoxin because of the improper storage of grain and, consequently, have a very high incidence of liver cancer. The research team measured cancer biomarkers in the urine, which were decreased by about half in people taking chlorophyllin supplements. Those results suggested that a simple and inexpensive intervention may help protect people from liver cancer caused by aflatoxin.

George is survived by Carol, his wife of 53 years, son Kelly, daughter Leanne Collis, sister Patricia Coffelt, and several grandchildren.

The Linus Pauling Institute will honor George through the establishment of the George Bailey Graduate Student Fellowship in Cancer Research and has set a goal of raising \$250,000 to endow this fellowship. "The students who receive this fellowship will be doubly inspired," said Balz Frei, LPI's director, "by those who have invested in their future, and by George's deep commitment to education, improved health, and rigorous scientific study."

Donations to the endowed fellowship in honor of George Bailey can be made by sending a check to: OSU Foundation, 850 SW 35th Street, Corvallis, OR 97333-4015; please write "George Bailey Fellowship" in the memo line. Alternatively, to make a gift online, please visit <http://giving.campaignforosu.org>, and select "Make a Gift" at the bottom of the page; please note "George Bailey Fellowship" in the box for "Area you'd like to support." **LPI**



Vitamin C and the Common Cold: Current Status

Alexander Michels, Ph.D. — LPI Research Associate

Vitamin C and the Common Cold by Linus Pauling was first published in 1970. In this book, he outlined his own experience with vitamin C and the reasons he believed gram doses of vitamin C would improve the immune system. He also reviewed the few clinical trials on vitamin C and the cold that had been published. The response to this book was enormous and long-lasting. Written at a time when natural therapies became increasingly more popular alternatives to conventional pharmaceuticals, the message resonated with the public with such intensity that vitamin C will forever be linked with the prevention and treatment of the cold.

From a scientific perspective, research studies on vitamin C and colds have often been poorly conducted and controversial. When Pauling wrote his book and advocated for the antiviral activity of vitamin C, there were only a few clinical studies that could be evaluated. Furthermore, scientific studies with ascorbic acid at the time were still in their infancy because the biological roles of ascorbic acid, such as its enzymatic functions and antioxidant activities, were poorly understood. In the decades since Pauling's initial review of the field, dozens of studies have been conducted and published on the topic—many inspired by Pauling's claims—but many still lack the level of sophistication needed to definitively study vitamin C in human subjects. Indeed, without a radical change in the experimental design of such studies and evidence from basic studies examining the potential mechanisms of action of vitamin C, we will not see scientific consensus on the effectiveness of vitamin C on the prevention and treatment of colds.

One of the issues that plagues supplement trials on the common cold is fairly fundamental: What is a cold? There are a whole host of viruses that cause symptoms associated with the cold, from rhinovirus and coronaviruses to parainfluenza. It is estimated that over 200 different types of viral infections can cause cold symptoms. It is difficult to detect an effect of vitamin C in clinical studies if it is only most effective against a subset of these viral infections. Furthermore, bacterial infections, especially of the respiratory tract, can induce symptoms that closely mimic a viral infection. The effectiveness of vitamin C supplementation in the prevention or treatment of bacterial infections of the respiratory tract is currently unclear.

In 1970, Pauling noted that “The mechanism of its [vitamin C] effectiveness against viral infection, such as a common cold, is not yet known.” He speculated that

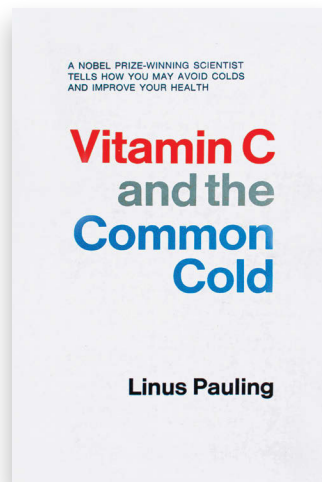
“the effectiveness of ascorbic acid in providing protection against viral diseases results from its function... in preventing the entry of virus particles into the cells.”

Even though more than 40 years have passed since Pauling wrote those lines, controlled laboratory trials with vitamin C and viral infection are exceedingly rare. As a consequence, we still do not know what dose of vitamin C might be effective at slowing or preventing many types of viral transmission in cells. However, Pauling and colleagues did identify some mechanisms by which vitamin C protected against HIV infectivity and replication in cell cultures, work that was published in the *Proceedings of the National Academy of Sciences* in 1990.

The data collected to date suggest that supplemental vitamin C most likely works by strengthening the immune system, as outlined in the Micronutrient Information Center on the LPI website. However, these data are controversial as they are often conducted in animal models that may poorly mimic the human immune system response.

Although Pauling advocated the use of multiple-gram doses of vitamin C per day for health with increasing doses to battle a cold infection, there are very few studies that attempt to follow this regimen. Doses used in clinical trials on vitamin C vary greatly and are likely a reflection of the uncertainty surrounding this field. Vitamin C is obtained from the diet, and nutritional status can play a large role in determining the amount of supplemental vitamin C that may have an influence. Similarly, comparisons are difficult to make with individuals who are deficient in vitamin C, insufficient (not yet saturating the plasma), or saturated (taking vitamin C in excess of that needed to saturate the plasma). Many studies do not make this distinction, as it is a concept few researchers fully understand.

In 2013, 70 years of clinical research on vitamin C and the cold were reviewed by Dr. Harri Hemilä for the Cochrane Database of Systematic Reviews. The results, which are summarized in the Linus Pauling Institute's Micronutrient Information Center, showed that regular supplementation with 200 mg or more of vitamin C reduced the duration of colds, with a greater benefit in children than in adults. In addition, regular vitamin C supplementation with 250 mg to 2,000 mg/day reduced the incidence of colds, but these results did not reach statistical significance except in those individuals under heavy physical stress (e.g., marathon runners, skiers, or soldiers in subarctic conditions).



Dr. Hemilä concluded that regular daily supplementation appears to be very important in reducing the severity and duration of colds. It is certainly possible that continuous supplementation with vitamin C for long periods prior to a viral challenge is important to ensure optimal immune system response, which takes time. A Japanese study, published in 2006 in the *European Journal of Clinical Nutrition*, deserves special mention in this regard. This study was a five-year trial designed to evaluate the effect of a daily dose of a low (50 mg) or high (500 mg) amount of vitamin C on the development of gastric cancer among 244 subjects. The researchers evaluated the effects of the vitamin C supplements on the common cold at the completion of the study and concluded that the risk of contracting three or more colds in the five-year period was decreased by 66% by the daily intake of the high-dose vitamin C supplement *versus* taking the lower dose of vitamin C.

However, it is important to note that these individuals in Japan were consuming a significant amount of vitamin C in the diet before the study started. Plasma measurements of ascorbic acid showed that these individuals were not deficient in the vitamin, and many had likely achieved vitamin C saturation of their blood. This could be why it took approximately two to three years of supplementation before the difference in cold incidence was apparent, covering many cold seasons in which subjects were likely exposed to repeated attacks by cold viruses. It is also possible that the effect of vitamin C supplementation is more effective against repeat infections, as it supports an activated immune system, rather than the first cold encountered after supplementation has begun.

The importance of plasma saturation was touched upon earlier this year in a study by Dr. Carol Johnston published in the journal *Nutrients*. This study was not included in Hemilä's Cochrane review but deserves special attention due to its sophisticated approach to the subject of vitamin C insufficiency. Unlike the majority of studies on vitamin C supplementation and the cold, including the Japanese paper described above, this study was limited to men who all had plasma vitamin C below 45 μM and were considered

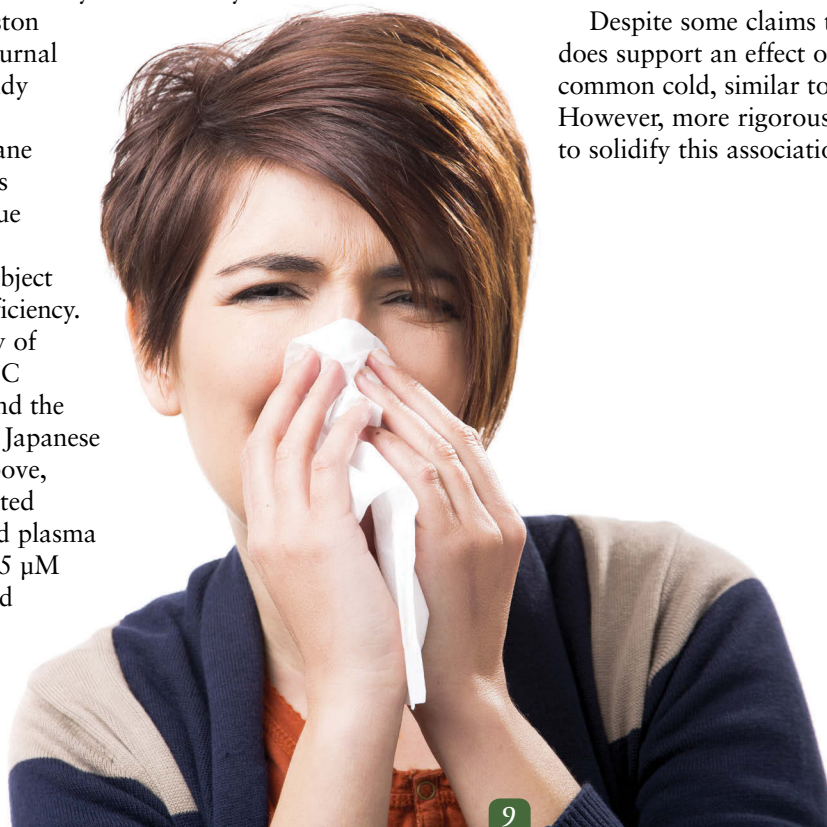
to have insufficient vitamin C status. During the eight-week trial, the vitamin C-supplemented individuals received 1,000 mg/day.

The vitamin C-supplemented subjects (but not those receiving placebo supplements) demonstrated elevated plasma ascorbic acid levels to near saturation. By the end of the study, the researchers found that subjects taking vitamin C supplements were 40% less likely to get a cold, and the duration of colds was decreased nearly 60%. In effect, this paper suggested that consuming a diet with inadequate vitamin C is possibly a factor in determining cold susceptibility. A greater and more immediate benefit on the common cold could be seen in individuals who correct inadequate vitamin C status with supplementation. The Linus Pauling Institute recommends that all adults consume at least 400 mg/day of vitamin C, which is a dose that will likely achieve plasma saturation in the population.

It's been proposed that vitamin C improves symptoms due to its antihistamine effect. While Dr. Johnston has conducted previous studies showing that vitamin C doses of 2,000 mg/day decreased blood histamine levels by 60%, doses of 1,000 mg/day in the recent study had no effect on histamine levels in blood.

It should be noted that supplementation taken only after the cold symptoms have begun, such as consuming vitamin C supplements at the first sign of a sore throat or runny nose, seems to have little appreciable effect. The beneficial effects of vitamin C supplementation on the common cold are more apparent when vitamin C is taken on a regular basis, when a cold is present or not, as Linus Pauling advocated. This is likely because of the amount of time taken for vitamin C to have its full effect on the immune system, as mentioned above.

Despite some claims to the contrary, the clinical evidence does support an effect of vitamin C supplements against the common cold, similar to Pauling's assessment 44 years ago. However, more rigorously-controlled studies are needed to solidify this association. **LPI**



The Linus Pauling Institute recommends that all adults consume at least **400 mg/day of vitamin C**, which is a dose that will likely achieve plasma saturation in the population.

New Discoveries in ALS



Dr. Joe Beckman

Dr. Joe Beckman has been working to understand ALS (amyotrophic lateral sclerosis or Lou Gehrig's disease) for over 20 years. ALS is characterized by the progressive loss of motor neurons, affecting all skeletal muscles and destroying the ability to move. The patients become completely paralyzed and usually die within one to five years of diagnosis.

Dr. Beckman and colleagues previously discovered the biological importance of an extremely reactive compound called peroxynitrite, a powerful oxidant and nitrating agent produced by immune cells that can kill pathogens but also damages biological molecules. Peroxynitrite is formed by reactions between superoxide and nitric oxide, which is involved in arterial dilation. Peroxynitrite induces motor neurons to die. Peroxynitrite also interacts with the antioxidant enzyme copper-zinc superoxide dismutase—an antioxidant protein that is sometimes mutated in ALS. When the copper-zinc superoxide dismutase loses zinc, the copper-containing enzyme becomes very reactive, generating peroxynitrite that can kill motor neurons. This observation led Dr. Beckman to try to compensate by feeding extra zinc to mice that have the defective superoxide dismutase and mimic ALS in order to normalize the zinc content in copper-zinc superoxide dismutase. That treatment slightly modified the progression of the disease, as did copper chelators that bind copper and remove it from the body.

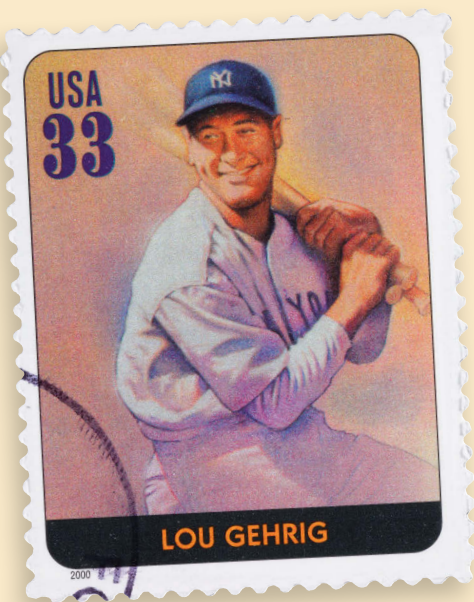
Dr. Beckman and his colleagues also found that a type of chemical

modification involving peroxynitrite—called nitration—to the amino acid tyrosine in the heat-shock protein Hsp90 is sufficient to cause that protein to change from a pro-survival protein to one that causes motor neurons to die. This discovery opened yet another perspective on ALS.

Most recently, Dr. Beckman was part of a team that found that treating the mice that mimic ALS with a copper compound (copper ATSM) paradoxically delayed progression of the disease and improved both their survival and locomotor function. This was surprising because giving additional copper to mice with the mutant zinc-deficient superoxide dismutase would be expected to increase the amount of the mutant enzyme—which it did—and, therefore, might be expected to increase motor neuron death and exacerbate the disease symptoms. In-depth investigation revealed that the copper compound was exquisitely targeted to cells in the spinal cord where it stabilized SOD in its mature and protective form. The researchers also found that much of the

superoxide dismutase in the spinal cord of the ALS mice was actually copper deficient. The key is that copper ATSM can deliver the right amount of copper to the right place in the spinal cord.

Copper ATSM has been used clinically for many years in cancer therapy to image tumors because it concentrates copper in tumor cells. The next steps are to more fully understand the role of copper in ALS and to conduct clinical studies to test safety and efficacy of copper ATSM in patients. **LPI**



Oxygen Club of California Annual Meeting

The Oxygen Club of California (OCC) held its annual meeting, co-sponsored by LPI, in Davis, California, in early May. *Oxidants and Antioxidants in Biology* convened 40 speakers from around the world to give presentations on “Nutrition and Redox Biology in Development and Health.” The conference was organized by LPI’s Maret Traber, who is the President of OCC; Patricia Oteiza; and Lester Packer.

The four sessions covered metabolic factors that influence development from conception to two years of age, flavonoids, redox signaling and systems biology, and vitamin E. Additionally, six posters were selected for oral presentations on Thursday afternoon.

Some highlights that may be of special interest to our readers:

The First 1,000 Days: From Metabolic Signal to Societal Impact



• **Mark Manary** (Washington University, St. Louis) noted that infants are born without microorganisms in their intestines. Each child develops an adult-like “microbiome”—the collection of microorganisms like bacteria, yeast, and viruses in the intestine—by the age of three. Every child’s microbiome is unique and

depends on manner of birth, breast-feeding, diet, and other environmental factors. The microbiota play an important role in immunity, food digestion, micronutrient synthesis, and detoxification.

• **Audrie Lin** (University of California-Berkeley) discussed the widespread problem of environmental enteropathy (pathological problems in the intestine) in poor, developing countries. Enteropathy—associated with undernutrition and poor sanitation—results in poor absorption of nutrients, the absorption of substances ordinarily not absorbed, and reduced efficacy of oral vaccines.

• The vital role of nutrition in early infancy was addressed by **Charles Stephensen** (University of California-Davis). Breast-feeding provides nutrients that ensure the development of immune-enhancing microbiota. If pregnant women are deficient in vitamin A, their infants will have impaired adaptive and innate immunity and an increased risk for death from infections.

• **Mulchand Patel** (The State University of New York-Buffalo) reported that nutritional stress during the fetal and suckling stage, which overlaps with organogenesis, can cause permanent changes in the structure and function of organs. Breast-feeding enhances immunity and cognition and reduces the probability of obesity later in life. Excessive calories from carbohydrates during infancy predisposes to obesity in adolescence and later.

Flavonoids: From Metabolism to Dietary Guidelines

• Flavonoids from fruit and vegetables have attracted much attention because of their *in vitro* antioxidant functions. **Alan Crozier** (University of Glasgow, UK) researched the pharmacokinetics (absorption and excretion) of epicatechin, a type of flavonoid found in tea, chocolate, grapes, and berries. He found that the concentration of epicatechin in plasma peaked twice after consumption: one hour later (representing absorption in the small intestine) and about seven hours later (representing absorption in the colon). Epicatechin was metabolized in the colon by microorganisms, and those metabolites, rather than the parent compound, were then absorbed. About 1.5% of the ingested dose was detected in plasma, with a biological half-life of one to three hours. Over 80% of epicatechin metabolites were excreted in urine within two days.

• **Christian Heiss** (University Düsseldorf, Germany) reported the results of the European FLAVIOLA study, designed to determine the impact of cocoa flavanols on cardiovascular disease risk. FLAVIOLA found that cocoa flavanol intake improved arterial flow-mediated dilation, lowered blood pressure, and reduced arterial stiffness in both genders across a broad age range. The beneficial effects were both acute and long lasting.

continued on page 12



• Anthocyanins are deeply pigmented flavonoids found in red and blue food like prunes, blueberries, and grapes. **Aedin Cassidy** (University of East Anglia, UK) informed us that their bioavailability—about 12%—(absorption into the blood stream) is greater than previously thought. The ingestion of food rich in anthocyanins is associated with reductions in blood pressure and reduced risk for heart attack, stroke, type 2 diabetes, and cardiovascular disease.

• **Jeremy Spencer** (University of Reading, UK) discussed the effect of flavonoids on cognitive performance. In one study, 500 mg of cocoa flavanols improved “executive” function—cognitive performance associated with attention—and memory processing. In another study, supplemental blueberries improved spatial memory in old rats. These effects are thought not to be due to antioxidant functions but rather to the inhibition of neuroinflammation and improved cell signaling, synaptic plasticity, and cerebrovascular blood flow.

• According to **Jiankang Liu** (Xi’an Jiaotong University, China) hydroxytyrosol, found in olives and olive oil, targets the mitochondria, resulting in improved function that affects aging and age-related disorders.

• **Zhaoping Li** (University of California-Los Angeles) explained that the absorption of pomegranate flavonoids called ellagitannins is highly complex. Ellagitannins are too large to be absorbed into the blood stream from the intestine, so they are broken down in the intestine to ellagic acid, which is then absorbed. Some ellagitannins are also metabolized by intestinal microorganisms to smaller compounds

called urolithins, which can be absorbed into the blood stream. Differences in the microbiotica among people affect the absorption of these compounds, illustrating the complexity of the process and difficulty in generalizing to whole populations.

Redox Signaling and Systems Biology in Health

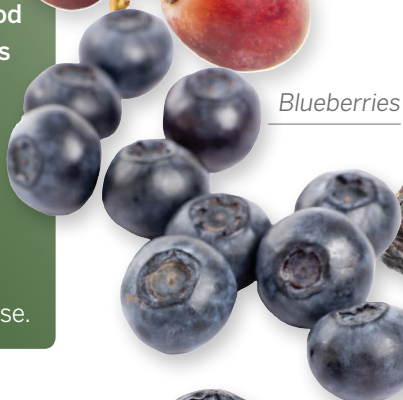
• Twenty-five proteins that incorporate selenium have been discovered. Their functions range from the metabolism of thyroid hormones to the control of protein folding. Some have antioxidant functions and offer protection against cancer. **Holger Steinbrenner** (Heinrich-Heine-University, Germany) emphasized that there is an optimum range of selenium levels in blood; lower levels may not afford disease protection, and very high levels may induce type 2 diabetes by affecting insulin secretion and signaling. The role of selenium in diabetes remains controversial because high glucose levels associated with diabetes may stimulate selenoprotein synthesis in the liver, thus explaining the association between high selenium levels in blood and diabetes.

• **Giuseppe Poli** (University of Turin, Italy) discussed the role of oxidized cholesterol in the development of Alzheimer’s disease. Head trauma, high blood pressure, diabetes, age, gender, family history, and hypercholesterolemia affect the risk for Alzheimer’s disease. While cholesterol itself does not cross the blood-brain barrier, its oxidation products—called oxysterols—are found in the brain. Oxysterols are very reactive and contribute to beta-amyloid production (a hallmark of Alzheimer’s disease) and inflammation. Another lipid peroxidation product—4-hydroxynonenal—has neurotoxic as well as pro-oxidant and pro-inflammatory properties. High levels of oxysterols are found in the cerebrospinal fluid of Alzheimer’s patients, and increasing levels of hydroxynonenal in the frontal cortex correlate with the severity of the disease. These findings may suggest strategies to prevent the generation of these toxic molecules.

• **Roberta Brinton** (University of Southern California, Los Angeles) presented information on the role of allopregnanolone, a neurosteroid synthesized from the hormone progesterone, in neurogenesis in the mouse brain. In cell culture and animal studies, allopregnanolone improved mitochondrial function. In mice, allopregnanolone decreased the amount of beta-amyloid in the brain, prompting current human trials to determine if allopregnanolone may be beneficial in cases of mild cognitive impairment or Alzheimer’s disease.



Grapes

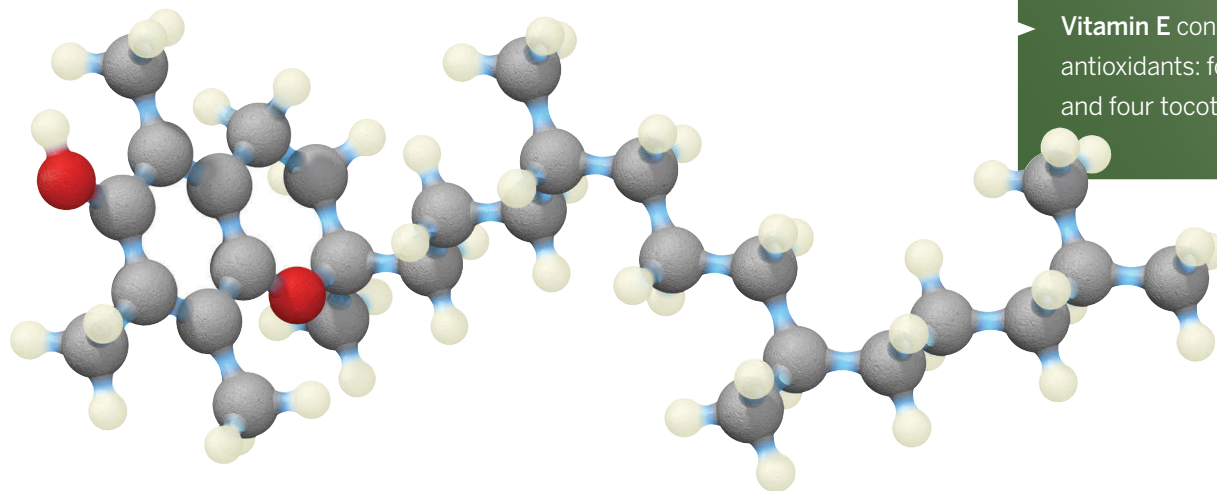


Blueberries



Prunes

The ingestion of food rich in anthocyanins is associated with reductions in blood pressure and reduced risk for heart attack, stroke, type 2 diabetes, and cardiovascular disease.



Vitamin E consists of eight antioxidants: four tocopherols and four tocotrienols.

Vitamin E: Tocopherol and Tocotrienols in Translational Medicine

- Vitamin E consists of eight antioxidants: four tocopherols and four tocotrienols. **Chandan Sen** (The Ohio State University, Columbus) noted that orally ingested tocotrienols are found in the brain in small concentrations that may protect against stroke injury. Clinical trials are under way to evaluate tocotrienols in secondary stroke prevention.

- **Arun Sanyal** (Virginia Commonwealth University, VA) noted that about 30% of Americans have steatosis—the abnormal accumulation of fat in the liver—that can lead to nonalcoholic fatty liver disease (NAFLD). Excessive fat in the liver increases lipid peroxidation (oxidative stress), inflammation, and mitochondrial dysfunction, and can cause liver fibrosis (scar tissue). In mice fed a high-fat diet to induce NAFLD, supplemental vitamin E favorably affected lipid metabolism in the liver and exerted anti-inflammatory effects. In a clinical trial with patients with fatty liver disease, supplemental vitamin E (800 IU of *d*-alpha-tocopherol daily for 96 weeks) improved several

important parameters (excluding fibrosis) and did not lead to the weight gain observed with drug therapy.

- Studies on the anti-inflammatory role of vitamin E have had conflicting results. **Joan Cook-Mills** (Northwestern University, Chicago) reported on studies in mice showing that two isoforms of vitamin E—alpha-tocopherol and gamma-tocopherol—have opposing effects on inflammation. Alpha-tocopherol inhibited, while gamma-tocopherol increased allergic lung inflammation. An analysis of human data obtained in research on cardiovascular disease risk found that increasing levels of alpha-tocopherol in blood were associated with better lung function, while increasing levels of gamma-tocopherol were associated with worse lung function.

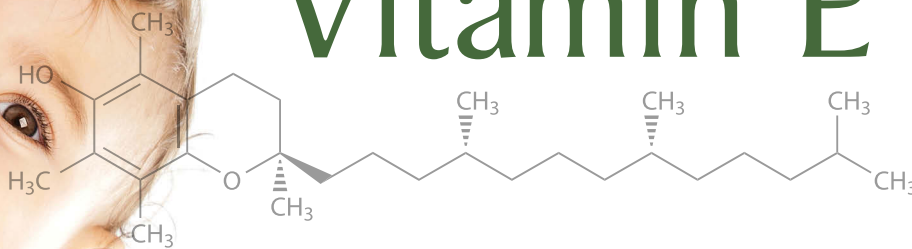
- Vitamin E deficiency is associated with muscle weakness and atrophy. **Paul McNeil** (Georgia Regents University, Augusta) found that vitamin E deficiency caused the failure of muscle repair in exercised rats. Oxidants inhibit repair mechanisms and thus contribute to the damage. Statins, widely prescribed to lower cholesterol, lower vitamin E levels in blood and may cause side effects, including muscle weakness. **LPI**

What is the Oxygen Club of California (OCC)?

OCC is a scientific organization dedicated to enhancing interactions and providing meetings and discussion forums worldwide to those interested in free radicals in biological systems, oxidants and antioxidants in biology and medicine, micronutrients, nutrition, and health.

OCC Mission: The OCC seeks to promote scientific interactions to improve the understanding of the roles of micronutrients in oxygen biology and medicine as they pertain to redox and energy metabolism, cell signaling and transcription, and epigenetics. An ultimate goal is to translate these findings to benefit human life- and health-span. OCC sponsors are from industrial organizations, health and medical foundations, government, and academia. We are also co-sponsored by the Linus Pauling Institute. With this wide support, conference organizers bring together the world's best researchers to address current scientific issues. The OCC provides a forum—in the form of prizes and awards—for the recognition of scientific achievements by both promising scholars and established investigators.

The Essentiality of Vitamin E



“Vitamin E is needed to protect the embryo’s brain before a woman knows she is pregnant. The vitamin E in a multivitamin is sufficient to meet the requirement.”

of frank vitamin E deficiency are extremely rare. People with severely impaired fat absorption may be at risk for deficiency symptoms because good absorption of fat-soluble vitamin E requires it be consumed with fat.

Vitamin E inadequacy, defined as very low concentrations of alpha-tocopherol in plasma, is associated with malnutrition in children. Inadequacy puts children at risk for infections, stunted growth, and anemia. Additionally, inadequacy during the first few years after conception can impair neurological development. As Dr. Traber said, “Vitamin E is needed to protect the embryo’s brain before a woman knows she is pregnant. The vitamin E in a multivitamin is sufficient to meet the requirement.” High vitamin E concentrations in infants at birth predict better cognitive function at two years of age.

Obese children are at increased risk for nonalcoholic fatty liver disease, which can lead to liver cirrhosis and cancer. Vitamin E supplementation in obese children (and adults) has improved the histological profile observed in fatty liver disease, probably due to reduced oxidative stress. Adequate consumption of vitamin E may inhibit the progression of fatty liver disease, which is dependent on lipid oxidation.

In mice and fish, vitamin E has been found to play a critical role in embryonic development, particularly the brain and nervous system. There are abundant fatty acids in the nervous system that are vulnerable to oxidation and dysfunction if vitamin E levels are inadequate. In older adults, vitamin E has been found to slow the progression of Alzheimer’s disease and to improve cognitive abilities, presumably by inhibiting the oxidation of fatty acids in the brain, especially DHA (docosahexaenoic acid)—an omega-3 fatty acid important in brain biochemistry and cognitive function.

Dr. Traber makes a convincing argument that we should be concerned about getting enough vitamin E, especially in the first 1,000 days after conception and when elderly. **LPI**

A recent review article on vitamin E inadequacy by LPI principal investigator Dr. Maret Traber has attracted a lot of attention. The supplemental intake of vitamin E has been controversial for many decades; early observational studies found an association between high vitamin E intake and reduced risk for heart disease, but subsequent studies have yielded mixed results, with some even claiming possible harm from supplemental vitamin E.



Dr. Maret Traber

The RDA of 15 mg (22.5 IU) of alpha-tocopherol, set by the Food and Nutrition Board of the Institute of Medicine in 2000, is based on the amount of vitamin E estimated to prevent the rupture of red blood cells, which could lead to anemia. This value was established for the intake of alpha-tocopherol – the only form of vitamin E among the eight forms of tocopherols and tocotrienols that is maintained in the body. Vitamin E is an important fat-soluble antioxidant that can be regenerated by vitamin C from its oxidized form in the body, as previously shown by Dr. Traber and her colleagues. Dr. Traber has published other articles asserting that the main, and perhaps only role of vitamin E in the body, is to prevent lipid oxidation.

While it is possible to consume 15 mg of alpha-tocopherol from the diet alone, about 90% of men and 96% of women do not meet this goal. Nuts and vegetable oils are good sources of vitamin E but may not be consumed daily in amounts sufficient to meet vitamin E adequacy. Despite this failure to reach the RDA of vitamin E, cases

DEVELOPMENTS

Gifts to LPI are an investment in your health

Since its founding in 1973, the Linus Pauling Institute (at OSU since 1996) has been helping people live longer, healthier lives, sharing information on the critical role of diet, micronutrients, and supplements in preventing and treating disease. Researchers at the Institute receive support from individuals, corporations and foundations, and grants from federal agencies, including the National Institutes of Health.

Earlier this year, *The New York Times* highlighted the role of philanthropy in advancing basic scientific research “Billionaires with Big Ideas Are Privatizing American Science.” With continued declines in federal funding, private donors are increasingly helping to shape science research through investment in areas of personal interest.

In the article, the *Times* noted that “spending on basic research has fallen by roughly a quarter, to \$30 billion last year, one of the sharpest declines ever,” resulting in fewer grants and—in some cases—research centers having to close their doors.

Research at the Linus Pauling Institute has not been exempt from the decline in federal spending, with

National Institutes of Health grants decreasing from \$5 million in 2009 to \$1.5 million last year.

Fewer research dollars from NIH means that gifts from individuals and corporate partners are having an even greater impact on our research. This year, LPI received significant gifts that are supporting a range of scientific studies: from better understanding of the causes and possible treatment for ALS, to the role of zinc in preventing inflammation, to the health benefits of xanthohumol, a flavonoid found in hops.

Gifts from people like you also are helping sustain the important research under way at the Institute. Last year, the Linus Pauling Institute received more than 1,300 gifts under \$10,000—gifts that together totaled more than \$215,000. These gifts were used to support research in the Institute, as well as outreach and education through the **Micronutrient Information Center** and the **Healthy Youth Program**.

Many thanks to all of you who already support the Linus Pauling Institute—your investment is making a vital difference in people’s lives. I invite all of you to consider a gift to the Institute and its mission of research and outreach, helping everyone live longer, healthier lives. **LPI**



Marlys Amundson
Director of Development

Photo by Jim Carroll Photography



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

Angela A. Capsouras	Susan B. Shearer
Matilda Goodman	Mark R. Sponenburgh
Eugenie M. Hagerman	Roberta Tumbiolo


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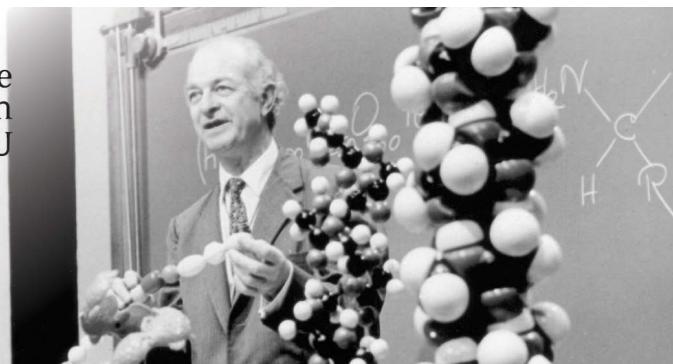
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