



OSU Oregon State University

# The Linus Pauling Institute

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



## From the Director

Balz Frei, Ph.D.  
LPI Director and Endowed Chair  
OSU Distinguished Professor of  
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In my last director's column (LPI Research Newsletter Spring/Summer 2010), I described my daily dietary supplement regimen, which consists of:

- A multivitamin/mineral with 100% of the Daily Value of most vitamins and essential minerals
- 500 milligrams (mg) of vitamin C
- 2,000 International Units (IU) (equaling 50 micrograms) of vitamin D<sub>3</sub>
- 1,200 mg of fish oil
- 400 mg of lipoic acid
- 1,000 mg of acetyl-L-carnitine

I also pointed out that I make sure to get 1,000 mg of calcium daily from my supplements and diet combined and that I currently don't take a vitamin E supplement because of concerns of an increased tendency to bleed when I take vitamin E together with a fish oil supplement.

I was pleased to see that my column inspired several articles in the popular media, including an upcoming article in *Vogue* magazine. My column also elicited feedback from my faculty colleagues and several LPI supporters around the country. One loyal donor from New York wrote me, "I do everything you do. So maybe that's why I just passed my 95<sup>th</sup> birthday." He continued, "Two things that I do that you left out, [but] I'm sure that you will add along the way: 1. Resveratrol 2. Coenzyme Q<sub>10</sub>." He was not the only LPI donor who asked about those two supplements.

In addition, Dr. Maret Traber, LPI Principal Investigator and one of the world's foremost authorities on vitamin E, wasn't quite happy that I preferred fish oil over her favorite vitamin. On the other hand, Dr. Donald Jump, an LPI

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## A New Twist on Vitamin C and Beer!

An Interview with Fred Stevens, Ph.D.  
Associate Professor of Medicinal Chemistry  
LPI Principal Investigator

**Q.** *You earned your Ph.D. in The Netherlands and spent some years as a post-doctoral research associate in Europe. You've been at OSU for about eight years and with LPI for the last five years. Is the academic research environment in Europe much different from what you find here?*

**A.** Of course, I've only seen it here at OSU as a faculty member. Here, we're much more focused on grant writing and getting money for research, while in Europe more time is spent on the research itself and writing papers.

**Q.** *How is academic research funded in Europe? Are there government agencies to which you apply for funding?*

**A.** In The Netherlands, the Ministry of Education allocates research funds to the universities, and they distribute that to the departments. Every department has state-funded professors, lecturers, technicians, and graduate students. At Oregon State University, only professors and instructors have state-funded positions. In The Netherlands there are opportunities to apply for national or European grants that are generally smaller in size.

**Q.** *What do you like to do when you're not working in the lab?*

**A.** I like to swim every day.

**Q.** *What's your position at LPI?*

**A.** I'm a Principal Investigator, and my academic home is the Department of Pharmaceutical Sciences in OSU's College of Pharmacy.

**Q.** *You also serve as co-director of the Biomolecular Mass Spectrometry Core Lab. What does that lab do?*

**A.** That lab provides mass spectrometry services for OSU. I provide expertise and instrumentation for analysis of

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— DESIGNATED BY THE NATIONAL INSTITUTES OF HEALTH AS A —

**Center of Excellence for Research on Complementary and Alternative Medicine**

Principal Investigator who is doing cutting-edge research on the biology and health effects of omega-3 fatty acids, including fish oils, didn't object. So, in this column, let me further comment on those three supplements—vitamin E, resveratrol, and coenzyme Q.

As you may know, vitamin E has taken a beating lately in both the scientific literature and popular press. Numerous large, randomized, placebo-controlled trials (RCT) of vitamin E supplementation for the prevention or treatment of heart disease or cancer have shown mixed and overall disappointing results. Some “meta-analyses” even suggested that vitamin E supplements may increase the risk of dying! Hence, the medical community, with a few exceptions, has all but dismissed any health benefit of supplemental vitamin E. However, these RCT, which are considered “the gold standard” for showing efficacy and safety of a medical drug, are far from being the ideal approach to test the efficacy and safety of micronutrient supplements like vitamins and essential minerals.

For example, while drugs are being recognized by the body as foreign substances (“xenobiotics”) that are rapidly metabolized, detoxified, and excreted, vitamins are essential for life (“vita” meaning life in Latin). Humans have evolved specific mechanisms to absorb and transport these essential nutrients to target cells and tissues for use in enzyme reactions and other biological functions. This also means that every person has been “exposed” to these micronutrients through their diet over their lifetime, which is not true for synthetic drugs that are only taken as part of the study. Therefore, RCT of micronutrients are *not* controlled: while the investigators give a placebo pill to the “control” group, all subjects in this control group have substantial levels of the vitamin to be tested in their bodies from the outset and throughout the course of the study! This severely limits the statistical power of the trial. For these and other reasons, the deck is stacked heavily against showing an effect of vitamin supplements in RCT.

A much better approach to evaluate the possible health benefits of vitamins is to look at the totality of evidence—test tube, cell culture, and animal studies, as well as epidemiological studies (human population studies) other than RCT. This totality of evidence shows that vitamin E acts as an important, fat-soluble antioxidant in our bodies and is very likely to have benefits in reducing the risk of diseases that have oxidative stress as a contributing factor, such as heart disease, ischemic stroke, Alzheimer's disease, and, possibly, certain cancers and Lou Gehrig's disease. Therefore, I firmly stand behind LPI's recommendation to take a daily supplement of 200 IU (133 mg) of natural source alpha-tocopherol (*d*-alpha-tocopherol) with a meal.

Coenzyme Q is often taken for two purposes: 1) to strengthen mitochondria, the “power plants” in our cells, and enhance energy metabolism; and 2) as an antioxidant to protect cell membranes and lipoproteins against oxidative damage. In fact, I co-authored two papers 20

years ago in *Proceedings of the National Academy of Sciences USA* showing that coenzyme Q is an effective fat-soluble antioxidant and protects low density lipoprotein—the “bad cholesterol” contributing to heart disease and stroke—more efficiently against oxidative damage than vitamin E. Coenzyme Q, unlike vitamins, is not an essential nutrient because it is synthesized in our bodies. A major problem with coenzyme Q supplements is that they are poorly absorbed, although some formulations are available that have increased bioavailability. It is also unclear whether supplemental coenzyme Q reaches its target tissues in the body, such as muscles, heart, and brain, especially in healthy individuals.

There is some evidence that coenzyme Q supplementation is useful as an adjunct to conventional drug therapy for congestive heart failure and may help in the treatment of other cardiovascular diseases and certain neurodegenerative diseases, such as Parkinson's or Huntington's disease. It's also possible, but not proven, that heart disease patients taking cholesterol-lowering statins may benefit from coenzyme Q supplementation. Statins are known to inhibit coenzyme Q synthesis in the body because they target the same pathway as cholesterol synthesis. Because of the limited overall evidence for benefit in healthy individuals, its low bioavailability, and the relatively high cost, LPI does not recommend regular coenzyme Q supplementation for healthy individuals. Certain subpopulations with specific diseases, older people, and individuals taking statin drugs may derive some benefit from coenzyme Q supplements.

Finally, resveratrol has been one of the hottest topics among the health-conscious public, as attested by the fact that the resveratrol article on LPI's Micronutrient Information Center (<http://lpi.oregonstate.edu/infocenter/phytochemicals/resveratrol>) is visited over 50,000 times each month. As we explain on that Web site, there is intriguing evidence that resveratrol “can inhibit the growth of cancer cells in culture and in some animal models” and may mimic caloric restriction and extend “the lifespan of yeast, worms, fruit flies, fish, and mice fed a high-calorie diet.” However, there is very little evidence for any health benefits of resveratrol supplements in humans, let alone evidence that it extends lifespan. The LPI research focus has been more on healthspan than lifespan, as we believe it is more important for people to attain a healthy old age than just a long life.

Commercially available resveratrol supplements contain doses that are hundreds or thousands of times higher than the amount naturally present in red wine or grapes. We don't know if taking such large doses of resveratrol over long periods of time is safe. Hence, at our current state of knowledge, the risk/benefit ratio seems unfavorable. LPI does not recommend resveratrol supplements, nor do I take them personally. I prefer to get my resveratrol the natural way. So, here's to your health! **LPI**



small organic molecules, which includes metabolites. We analyze metabolites made from lipid peroxidation products and xenobiotics. We do a lot of work for people in the Colleges of Pharmacy and Veterinary Medicine, analyzing drugs and case studies.

**Q. You worked for many years on a phytochemical called xanthohumol found in beer. What is xanthohumol and what does it do in cells?**

**A.** Xanthohumol is a flavonoid found in hops. We've known about it for a long time, but it never received any attention from beer brewers because it has no taste. I got interested in xanthohumol back in 1995 when I became a post-doc at Oregon State University with Professor Max Deinzer. We were investigating the chemistry of hops and focused on xanthohumol, which we were able to isolate. Professor Don Buehler, Val Miranda, and I investigated the activity of xanthohumol in cancer cells and found that it induces phase 2 enzymes—enzymes that detoxify carcinogens. That is good for cancer chemoprotection. We also found that xanthohumol inhibits phase 1 enzymes that activate pro-carcinogens. So the effect is two-fold: xanthohumol prevents activation of pro-carcinogens into carcinogens by phase 1 enzymes and helps detoxify carcinogens by the phase 2 process. We can clearly say that xanthohumol is a cancer chemoprotective compound in cell culture.

**Q. One problem with the cell culture model is that it doesn't necessarily predict what will happen in the body because compounds need to be absorbed into the blood stream to have many physiological effects. Have you studied the bioavailability of xanthohumol—how much actually gets into the blood stream?**

**A.** We have measured pharmacokinetics in a handful of people and found that xanthohumol is absorbed and may be distributed to the tissues, which seem to hold onto it for a long time. It has a long half-life in the body. Although the plasma levels are relatively low, tissue levels may be much higher. Low plasma levels don't necessarily imply a lack of biological activity.

**Q. Does xanthohumol act as an antioxidant in the body?**

**A.** It has antioxidant activity *in vitro*. We don't think that it functions as an antioxidant in the body.

**Q. Is xanthohumol found in other dietary plants or in lagers and wines?**

**A.** It is found in any beer that is produced from hops but not in dietary plants. It's not found in beers that are made from hop extracts.

**Q. One of the hop flavonoids you studied, 8-prenylnaringenin, is a potent phytoestrogen. How may that affect health?**

**A.** Prenylnaringenin was identified as a metabolite of isoxanthohumol, which is the isomer made from xanthohumol when you brew the beer. However, the

level of prenylnaringenin in beer is very low, and it's very doubtful that the low amount in beer would be estrogenic in people. In the last couple of years, studies have shown that some people have certain microorganisms in the gut that are able to convert isoxanthohumol into prenylnaringenin. Xanthohumol supplements in those people may be problematic, but the amount in beer is not a concern.

**Q. Is there any way to determine which people have microorganisms that convert isoxanthohumol to prenylnaringenin?**

**A.** There is no easy test to identify these people.

**Q. How might xanthohumol help prevent prostate cancer?**

**A.** We published a paper with Emily Ho, another LPI Principal Investigator, showing that xanthohumol interferes with NFκB signaling. NFκB is a protein and master switch for the regulation of about 400 genes that are involved in inflammatory processes. Xanthohumol binds to a certain chemical group in a structural subunit of NFκB and prevents its signaling, thereby blunting or reducing the inflammatory response. NFκB-driven inflammation is an important process in the development of prostate cancer.

**Q. You studied another kind of flavonoid, proanthocyanidins. What foods contain these?**

**A.** Virtually all plants make proanthocyanidins, also called condensed tannins. They are found in hops, grapes and their seeds, apples, cocoa, tea, and many fruits. Anything that has an astringent taste probably contains a lot of proanthocyanidins.

**Q. How do proanthocyanidins affect the risk for colorectal cancer?**

**A.** We found that proanthocyanidins kill cancer cells *in vitro* by producing hydrogen peroxide. Bioavailability, or absorption into the blood stream, is not an issue because the colorectal cells would be directly exposed to ingested proanthocyanidins.

**Q. Why are xanthohumol and proanthocyanidins synthesized by plants?**

**A.** Well, a lot of phytochemicals are defense chemicals against herbivores. Of course, unripe fruits contain a lot of tannins and are not palatable to animals, so they don't eat them until the fruit is ripe. Then the animals consume the fruit and disperse the seeds, which helps ensure survival of the plant species.

**Q. For the last half-dozen years, you have worked on reactions between vitamin C and rancid fats, or lipid peroxidation products, also called LPO products. How does vitamin C react with these?**

**A.** Well, this is a difficult problem. When fats get oxidized, they make degradation products known as reactive aldehydes.

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**Q. Does that happen in the body?**

**A.** Yes, we have lots of evidence that these aldehydes are made in the body. Vitamin C can react *in vitro* with these compounds to make what we call ascorbylated lipids. This has been shown for a very small lipid peroxidation product called acrolein, which reacts with vitamin C in cultured cells. This is an important finding because it shows that vitamin C can compete with a well-known pathway called glutathione conjugation that helps get rid of toxic products. At first we were not very successful in finding these LPO products. It now appears that the ascorbylated aldehydes get further metabolized. We have identified one metabolite and want to find out if it is the final end product or gets further modified. Once we have clearly identified the end products, we will try to determine if this process happens in the body.

**Q. What is the physiological significance of LPO products? How are they detrimental to health?**

**A.** Not all of them are bad. Prostaglandins are enzymatic breakdown products of lipid hydroperoxides. Prostaglandins are ubiquitous and have very important functions, including the regulation of platelet activity and inflammation. On the other hand, nonenzymatic lipid peroxidation generates reactive aldehydes that can react with DNA in a nonspecific manner. This can cause DNA mutations that could lead to cancer. Aldehydes can also react with proteins, including enzymes, in a nonspecific manner, causing damage that can lead to cellular dysfunction. For example, elevated levels of the LPO product, 4-hydroxy-2-nonenal (HNE), have been associated with the development and progression of Alzheimer's disease.

**Q. Can LPO products be used as biomarkers to measure oxidative stress in the body?**

**A.** We have done three studies on this. The first was a study where we exposed rats to carbon tetrachloride, which is a strong inducer of lipid peroxidation and oxidative stress. We then looked in the urine for metabolites of HNE and a related product called 4-oxo-2-nonen-1-ol (ONO). We were the first group to find evidence for *in vivo* production and metabolism of ONO. It certainly has value as a biomarker of oxidative stress in rats. In the second study, we used smokers as a model of chronic oxidative stress in humans. We found that the smokers had higher levels of LPO products, but the differences were not statistically significant because people vary widely in levels of oxidative stress. Then we looked at smokers who stopped smoking and found that smoking cessation results in a substantial decrease in these biomarkers. With LPI's Maret Traber, we wanted to know if vitamin C supplementation would affect these new biomarkers. Using mass spectrometric analysis of the subjects' urine, we found that vitamin C supplementation decreases these HNE and ONO metabolites.

**Q. Would these be better biomarkers of oxidative stress than F<sub>2</sub>-isoprostanes or other standard biomarkers?**

**A.** The biomarkers that we measure are present in much higher concentrations, so that's a benefit analytically. Also, some of them are very stable because they have been fully oxidized—they are final end products easy to work with. Although this work is still in the very early stages, we can say that in the study we just completed vitamin C supplementation leads to a decrease in HNE metabolites, whereas F<sub>2</sub>-isoprostanes did not change.

**Q. You have studied a ubiquitous chemical called acrolein that is formed by cooking food, hydrocarbon combustion, cigarette smoking, and other processes. What does acrolein do in the body?**

**A.** HNE is the prototypical lipid oxidation product, but acrolein can also be formed from lipid peroxidation. Its major source is smoking. A study a couple of years ago claimed that acrolein—not tar—is the carcinogenic compound in cigarette smoke. That, of course, received a lot of interest in the media.

**Q. What epidemiological evidence links acrolein to lung cancer?**

**A.** Acrolein is a very small molecule and a volatile substance, so when you cook with vegetable oils, it's probably present in the kitchen air. In Hong Kong, wok cooking in unventilated kitchens, not smoking, is thought to be the cause of the high rate of lung cancer in women.

**Q. How does vitamin C affect acrolein?**

**A.** Acrolein is a reactive aldehyde that can damage bio-molecules. Vitamin C can function chemically as an electron donor. Acrolein is an acceptor, and they react with each other to form an adduct, ascorbylated acrolein. It is a very efficient reaction and is easily done in the test tube. We examined the crystal structure of ascorbylated acrolein with a chemist here at OSU. Since vitamin C adduction to acrolein happens so easily *in vitro*, we think that this reaction may have biological significance *in vivo*. Of course, the metabolism of this compound needs to be further studied. When we started, Nicholas Kesinger, my graduate student, challenged cells with acrolein but didn't find anything unusual. That was very disappointing because we had hoped to detect evidence of the chemical reaction we expected. Then we added synthetic ascorbylated acrolein to cells, and it disappeared, although we could easily measure it in a buffer solution. So then we started thinking that ascorbylated acrolein might be metabolized and we discovered that it follows a very unusual metabolic pathway. That was the main finding of Nick Kesinger's Ph.D. project that he completed with me in LPI.

**Q. So vitamin C forms an adduct with acrolein that then further degrades through metabolic processes in the body, rendering the acrolein harmless.**

**A.** Right.

**Q. Have reactive aldehydes been found to cause diseases in humans?**

**A.** A possible example is Alzheimer's disease. There are a lot of studies showing the involvement of HNE in the pathogenesis of Alzheimer's disease, and, as I mentioned, acrolein is involved in lung cancer in smokers.

**Q. How does HNE contribute to Alzheimer's disease?**

**A.** Scientists think that HNE damages proteins that are important for prevention or delaying onset of the disease. For example, HNE can inactivate an enzyme called neprilysin that breaks down the amyloid beta plaques in the brain of Alzheimer's patients.

**Q. You've described a reaction between vitamin C and certain phytochemicals that you call ascorbylation, which you've already discussed. How is this relevant to health?**

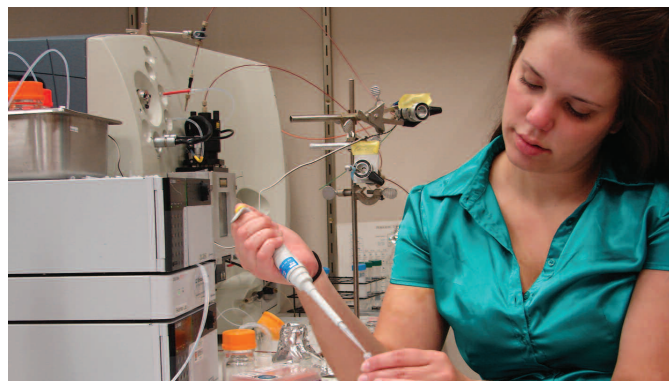
**A.** This is very controversial because many scientists claim that electrophilic products—chemicals attracted to electron donors—in the body interact with glutathione. The idea that vitamin C plays a role as a nucleophile came from nature. A nucleophile has an excess of electrons—it's an electron donor—and combines with substances that have a deficiency of electrons to make an adduct. About 10 years ago, I isolated a natural product from henna, which contains a dye that's a naphthoquinone. The naphthoquinone reacts readily with vitamin C in the plant material when you crush it to make the dye. That's how I found my first ascorbylated natural product. So I thought, if this reaction happens so readily in plant material, it must have some biological significance. Then we searched more carefully in the plant kingdom for the specific structural moiety allowing the ascorbylation reaction, and to our surprise, we found 33 natural products that had that moiety. In most cases, the authors were not even aware that they had a vitamin C adduct.

**Q. Are these adducts formed only in plants, not in the body during digestion or metabolism?**

**A.** Right. These are true natural products. A famous example is ascorbigen, which is an indole-3-carbinol metabolite with vitamin C found in cabbage. There are also tannins that contain the vitamin C moiety. For example, a tea flavonoid—epigallocatechin gallate or EGCG—has been found in an ascorbylated form. There are many, many examples. There must be a really significant reason for this reaction in nature, and that is what we are trying to understand.

**Q. Those ascorbylated compounds occur in plants and, of course, may or may not have much relevance to human physiology or health.**

**A.** Right. Plants make vitamin C for their own health; they don't make it just for us! I don't know the relevance of ascorbylation for plants, but if it reduces the reactivity of waste products in the human body, then it could be considered a detoxification pathway.



**Q. Do ascorbylated compounds contribute to the antioxidant capacity of food?**

**A.** No, because the moiety has been totally modified. Vitamin C has a double bond and two hydroxyl groups. Basically, it functions as an antioxidant because it's a one-electron donor. The ascorbylation reaction is a two-electron reaction, and then the double bond in the vitamin C molecule disappears, and the antioxidant activity is lost.

**Q. Where is your research headed in the next few years?**

**A.** We will continue to study the interaction between vitamin C and reactive aldehydes. There are multiple ways in which vitamin C may be helpful in unexpected ways. Last year we compared levels of protein adduction by HNE in human monocytes *in vitro* with and without vitamin C. We found that vitamin C reduced the level of protein adduction by HNE by 30%. We couldn't attribute all of that to the formation of the ascorbylated products because we didn't know about the metabolism at the time. Nevertheless, there must be other mechanisms by which vitamin C protects proteins from damage by these reactive aldehydes. This could be very important for a number of diseases. We're working on cardiovascular disease, and in the future we will determine the effect of vitamin C in protecting proteins that are important in cardiovascular health.

**Q. What kinds of proteins?**

**A.** One enzyme we are studying is aldehyde dehydrogenase. This is an enzyme that metabolizes HNE, but HNE can also damage the enzyme. Of course, if HNE damages the enzyme, then HNE itself cannot be metabolized and more and more of it is accumulated and creates more and more damage. If vitamin C could protect the enzyme, then HNE could be properly metabolized, and there would be less damage.

**Q. LPI will move into our new building, the Linus Pauling Science Center, next summer. To which research cluster will you belong?**

**A.** I will be with Balz Frei, Maret Traber, and Don Jump in the cardiovascular and metabolic diseases group.

**Q. You must be looking forward to that.**

**A.** I'm very excited! **LPI**



## Induction of Apoptosis in Cancer Cells

Siva Kumar Kolluri, Ph.D.  
OSU Assistant Professor  
Department of Environmental and  
Molecular Toxicology

*Summary: To effectively treat cancer, we want to protect normal cells and selectively kill cancer cells. A protein called Bcl-2 regulates apoptosis or cell death. An abundance of Bcl-2 produced in cancer cells prevents apoptosis. Prevention of apoptosis promotes cancer cell growth. Another protein called Nur77 can interact with Bcl-2 and convert Bcl-2 from an anti-apoptotic to a pro-apoptotic protein in cancer cells. We discovered that small fragments of Nur77 (peptides, or groups of amino acids that make proteins) could also interact with Bcl-2, leading to the death of cancer cells.*

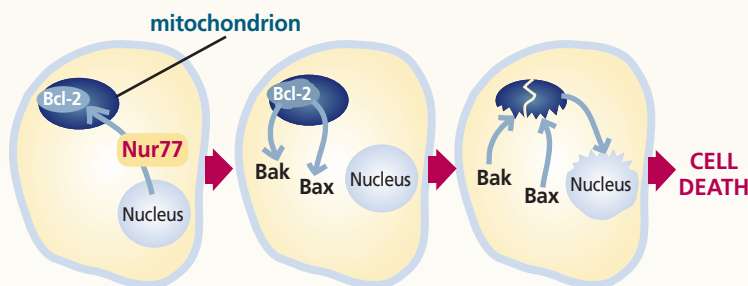
Damaged cells in the body are eliminated through a process involving cell suicide known as apoptosis. Since apoptosis normally eliminates cells with increased malignant potential, such as those with damaged DNA or aberrant cell cycling, inhibition of apoptosis is highly important to tumor initiation and progression. All cells contain both pro-death and pro-survival proteins that regulate the process of cell apoptosis. These pro-survival proteins are abundantly expressed in cancer cells, which help them to thrive and resist elimination upon treatment with anti-cancer drugs. Bcl-2—an acronym for B-cell lymphoma 2—is one such survival protein that is overexpressed in a variety of cancers, including leukemia and lung, liver, gastric, ovarian, prostate, and breast cancers.

Members of the Bcl-2 family of proteins modulate apoptosis in different cell types in response to various stimuli. Some of these members, such as Bcl-2 and Bcl-X<sub>L</sub>, act to inhibit apoptosis (anti-apoptotic), while others, such as Bax, Bak, Bid, and Bad, promote apoptosis (pro-apoptotic). All members of the Bcl-2 family possess at least one of the four structural domains called Bcl-2 homology (BH) domains. Domains are discrete, stable components of protein structure that combine in different ways to make different proteins. Proteins that contain only one BH domain (BH3) are pro-apoptotic. Interestingly, anti-apoptotic Bcl-2 members contain this BH3 domain along with three other BH domains (1, 2 and 4). To prevent apoptosis, anti-apoptotic members bind pro-apoptotic members containing only the BH3 domain, and while this occurs in normal cells, a significant upregulation of Bcl-2 in cancer cells disrupts the normal balance, allowing cancerous cells to survive.

Nur77 is a protein that belongs to the superfamily of nuclear receptors that controls gene expression in cells. Nur77 has the capacity to not only regulate gene expression but also to induce apoptosis. Importantly, the apoptotic effect of Nur77 family members is clinically relevant, since the expression of the Nur77 subfamily member Nor-1 improves survival in diffuse, large B-cell lymphoma patients treated with chemotherapeutic drugs. Gene expression profiles have also revealed that downregulation of Nur77 expression is associated with metastasis of primary solid tumors.

We had previously demonstrated a new paradigm in cancer cell apoptosis, whereby Nur77 moves from the cell's nucleus to its mitochondria, where it interacts with Bcl-2, converting this normally anti-apoptotic protein into a pro-apoptotic protein that induces cell death. While trying to understand this unexpected phenomenon, we discovered that Nur77 causes an alteration in the Bcl-2 structural conformation that exposes its hidden pro-apoptotic BH3 domain.

In collaboration with Drs. Xiao-kun Zhang, Arnold Satterthwait, John Reed, and other colleagues at the Sanford-Burnham Medical Research Institute, we discovered that even short protein fragments or peptides from Nur77's Bcl-2 binding region are able to induce the same conformation changes in Bcl-2 that Nur77 induces. We found that the Nur77-derived short peptides bound to Bcl-2 and exposed its BH3 domain, which allowed for Bcl-2 to activate two pro-apoptotic proteins, Bax and Bak, to initiate apoptosis. Importantly, the altered Bcl-2 also neutralized the anti-apoptotic activity of other Bcl-2 family members. We then found that these peptides induced apoptosis in cancer cells *in vitro* and suppressed the growth of human cancer cells explanted in mice. To further confirm this relationship, we established that the pro-apoptotic activity of the Nur77 peptides was dependent on Bcl-2 expression.



How Nur77 initiates apoptosis in cancer cells

A majority of solid tumors are protected from apoptosis by overexpression of Bcl-2. Accordingly, there have been efforts in the last few years to identify compounds that neutralize Bcl-2's anti-apoptotic functions to make cancer cells more susceptible to chemotherapeutic drugs or compounds. This has led to the development of approaches that either decrease Bcl-2 protein expression or neutralize the survival functions of Bcl-2 with small molecules. However, neutralizing Bcl-2's normal anti-apoptotic/pro-apoptotic function alone may not be sufficient, since other anti-apoptotic proteins must also be inhibited for the therapeutics to be effective. Importantly, our studies have established that Nur77 peptides or small molecule mimetics not only inhibit Bcl-2 survival function but also neutralize other Bcl-2 survival proteins by altering Bcl-2 conformation. This will likely have therapeutic value in treating a variety of cancers.

Pilot project funding from LPI helped us to conduct the experiments that reveal the mechanism of cell death induced by the converted Bcl-2. Furthermore, the LPI pilot project award helped us to establish assays to potentially identify additional compounds that convert Bcl-2 from a cell protector to a cell killer. This research helped to secure additional funding from the Breast Cancer Research Program of the Department of Defense. **LPI**





## Developments

*Michele Erickson  
LPI Director of Development*

**I**t is with great enthusiasm and commitment that I introduce myself as the new Director of Development for the Linus Pauling Institute. It's an honor for me to work on behalf of LPI's Director, Balz Frei, and all the other investigators who are conducting such innovative and important nutrition research. I am eager to work with all of you who have given so much to ensure the outstanding reputation and scientific progress of LPI.

### A Vision to Expand LPI's Healthy Aging Program

The Linus Pauling Institute has been conducting research and providing education for nearly 40 years to help people everywhere achieve healthy, productive lives and to prevent disease through diet, dietary supplements, and lifestyle. LPI's internationally recognized expertise in orthomolecular or preventive medicine and world-class faculty have positioned it as a leading international research institute. Donors like you propelled this work by contributing more than \$33 million to the Linus Pauling Institute in the last few years, reaching our goal to fund construction of the new Linus Pauling Science Center and initiating the creation of LPI's Healthy Aging Program by endowing a Chair for the program director and recruiting the first of three additional faculty to work on age-related health issues, such as declining immune function and Alzheimer's or Parkinson's disease.

Increasingly, people are turning to the Linus Pauling Institute for expert, science-based advice on the "protect and prevent" role of micronutrients and other dietary factors vital to healthy aging. When fully realized, LPI's vision of five world-class Principal Investigators leading the Healthy Aging Program's research efforts, leveraged by a dedicated team of graduate students and post-doctoral fellows, will allow us to make tremendous strides. Already designated by the National Institutes of Health (NIH) as one of the nation's first two Centers of Excellence for Research on Complementary and Alternative Medicine, the Linus Pauling Institute will be highly competitive for NIH grants and influential as advisors on national committees shaping future research and recommendations for improving health and wellness in the U.S.

### Donor Contributions Lead to Success

The advancement of the Linus Pauling Institute has been extraordinary. Construction of the Linus

Pauling Science Center is a visible and tangible indication of LPI's growth and will enhance scientific research, extramural funding, and public education to help use this knowledge for people to live healthier lives and reduce suffering. LPI's recent accomplishments:

- Linus Pauling Science Center construction (Grand Opening summer 2011)
- The J. Burgess and Elizabeth B. Jamieson Endowed Chair in Healthspan Research
- Two new Principal Investigators in immuno-senescence/vitamin D research and cancer chemoprotection, respectively
- Funding for another new Principal Investigator in the Biochemistry of Aging and commencement of an international search
- Expansion of LPI's online Micronutrient Information Center, which receives over 25 million hits every year

While these accomplishments are extraordinary, **it is the annual gifts from dedicated donors like you who keep the important day-to-day research going.** Thank you for the vital gifts you have made to LPI to sustain our Research Innovation Fund. Please continue your essential support. Together, we can help people everywhere live longer and healthier lives.

I hope to be able to meet with many of you in person and hear about your experiences. Please don't hesitate to contact me in the office at 541-737-3744 or by email at [Michele.Erickson@oregonstate.edu](mailto:Michele.Erickson@oregonstate.edu) if I can be of assistance in any way.

P.S. Construction remains on schedule for the Institute's new home, the Linus Pauling Science Center (see <http://webcam.oregonstate.edu/lpsc/>)!

There's still time to name a room by designating LPI in your estate plans. For information, please contact me by phone or email. **LPI**

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

John F. Holterhoff	Grace Telenius Davis
Nancy J. Bradford	Helen P. Rumbel
Jean S. White	Nancy Ann Finke Bower
Gerald D. Carney	Mildred G. Mayne
Mavis E. DeCius	William R. Town



## The LPI Fitness & Nutrition Study in Children

*Simone Frei, MPH, MA  
LPI Program Coordinator*

Childhood obesity has become epidemic in the United States. Results from the 2007-2008 National Health and Nutrition Examination Survey estimate that 16.9% of children and adolescents aged 2-19 years are obese compared to only 5% in 1971. According to the 2007 National Survey of Children's Health, obesity rates for 10- to 17-year-old children range from 9.6% in Oregon to 22% in Mississippi.

Childhood obesity is associated with many health risks, such as pediatric hypertension, type 2 diabetes, orthopedic complications, and impaired mobility, and can result in psycho-social consequences like low self-esteem, social isolation, and discrimination. In addition, long-term studies have shown that obese children are likely to become obese adults. Most experts agree that physical inactivity and a poor diet are the root causes of obesity and that there is a widespread need to promote regular physical exercise and better eating habits among children. Clinicians and public health officials recommend that children engage daily in 60 minutes of moderate to vigorous physical activity, such as brisk walking, running, cycling, or playing outdoors. However, opportunities for children to be physically active during school days have decreased in most schools across the United States. Additionally, time spent on sedentary activities like watching TV or playing computer and other video games is increasing.

Several surveys indicate that many children do not obtain an adequate intake of many vitamins and minerals in their diet that are essential for good health and disease prevention. The Multivitamins & Public Health Workshop in 2003

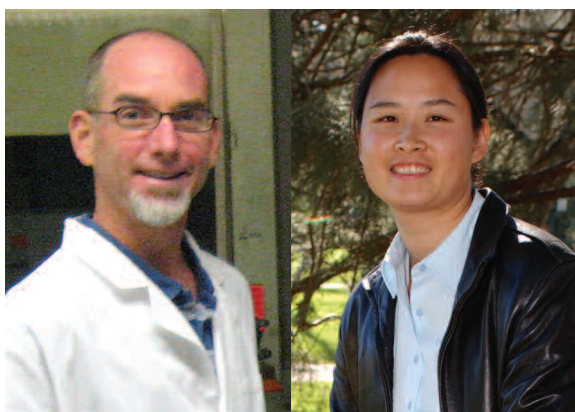
suggested that "multivitamin/mineral supplementation to increase micronutrient intake to recommended levels is a prudent, inexpensive, and convenient way to improve the public's health status."

Vitamin D deficiency in American children is becoming a major concern. While parents and pediatricians may have assumed that children are getting enough vitamin D from sunshine, vitamin D-rich foods, and vitamin D-fortified milk, a study published in 2009 found that 70% of children have inadequate vitamin D levels and nearly 10% are deficient. Most people living above 40 degrees latitude do not obtain enough vitamin D from about mid-November to early March. People with dark-colored skin have a significantly lower ability to synthesize vitamin D from sunlight, as do the elderly, and obesity increases the risk of vitamin D deficiency because these individuals cannot easily access the vitamin D stored in their body fat.

New evidence shows that vitamin D deficiency may not only be associated with rickets and osteoporosis but also with muscle pain and weakness; cancers of the breast, prostate, and colon; high blood pressure; heart disease; multiple sclerosis; rheumatoid arthritis; osteoarthritis; type 1 and 2 diabetes; irritable bowel syndrome; and Crohn's disease. Research by LPI's Dr. Adrian Gombart has shown that vitamin D also plays a critical role in immune function by enhancing the production of a protein in the body that can fight viruses and bacteria (see the Spring/Summer 2010 LPI Research Newsletter). Hence, vitamin D plays an important role in preventing upper respiratory tract infections, tuberculosis, and other viral and bacterial infections that occur during the flu season. Based on these findings, the Linus Pauling Institute recommends that children supplement their daily diet with 1000 IU of vitamin D to achieve good health.

## The LPI Fitness & Nutrition Study

During the 2009-2010 school year, we implemented a program to increase fruit and vegetable consumption and physical activity among elementary school children in the



Scott Leonard

Emily Ho

## Recognition for LPI Staff and Faculty

At the recent OSU University Day, two members of LPI were recognized for their outstanding achievements. **Scott Leonard** of Dr. Maret Traber's lab in LPI was honored with the Outstanding Faculty Research Assistant Award, which recognizes "scholarly achievement and a level of innovation and effort that far exceeds expectations" and truly exceptional performance. LPI Principal Investigator **Dr. Emily Ho** was given the Promising Scholar Award, which recognizes "outstanding scholarly activity that represents significant intellectual work validated and communicated through peer review." She will hold the title of "Promising Scholar" for 2011.



3<sup>rd</sup>, 4<sup>th</sup>, and 5<sup>th</sup> grades at a school in Corvallis, Oregon. Three times a week, students participating in the program walked or ran on an outdoor track during the 20-minute morning recess; ate a fresh fruit for morning snack instead of the usual crackers, chips, or cookies; and ate fruits and/or vegetables with lunch. Students were also offered a daily children's multivitamin/mineral with 1000 IU of vitamin D. Lastly, we provided three modules of nutrition education classes during the school year.

We set up a point system to assess study compliance. Each student could collect a maximum of 9 points per week (3 points each for walking/running, fruit snack, and fruit and vegetables for lunch); a minimum of 6 points per week was required (66% participation) to be considered compliant and remain in the study. Incentives, such as T-shirts and Frisbees, were given to enhance compliance. We measured the students' height and weight and calculated their body mass index (BMI) three times during the school year and asked them to fill out a validated Food Questionnaire, Physical Activity Survey, and Pediatric Quality of Life Questionnaire twice during the school year.

One hundred and thirteen students, or 52% of the 219 students in grades 3-5 at the school, voluntarily enrolled in the study. Interestingly, 71% of all girls in grades 3-5 enrolled, but only 37% of boys enrolled. Of the 113 enrollees, 85 completed the study, corresponding to 75% compliance. Among these 85 students, the level of participation differed significantly between grades—3<sup>rd</sup> graders had a higher participation level (84%) than the 4<sup>th</sup> and 5<sup>th</sup> graders (70% and 69%, respectively). The level of participation for all grades decreased from 82% at the beginning to 70% at the end of the study. Further analysis showed that the decline in participation was due to a decrease in walking/running during recess, whereas fruit and vegetable consumption remained unchanged. This declining participation in physical activity was observed for all grades, but the 3<sup>rd</sup> grade students maintained a significantly higher level of participation than the 4<sup>th</sup> and 5<sup>th</sup> graders throughout the study.

The calculation of body mass index (BMI) for children, unlike adults, takes into account the child's gender and age and is expressed as a percentile. A BMI below the 85<sup>th</sup> percentile indicates a healthy weight; overweight is defined as a BMI between the 85<sup>th</sup> and 95<sup>th</sup> percentile; and obese is a BMI higher than the 95<sup>th</sup> percentile. Among our study's students, the number of overweight and obese students decreased with a corresponding increase in students with a healthy weight (see figure at top right). This reduced prevalence of overweight and obesity was mainly attributable to a decrease in the mean BMI of the children in the three 3<sup>rd</sup> grade classrooms and in one 5<sup>th</sup> grade classroom. The mean BMI of the 3<sup>rd</sup> graders decreased from the 62<sup>nd</sup> to the 51<sup>st</sup> percentile and for the 5<sup>th</sup> graders from the 71<sup>st</sup> to the 64<sup>th</sup> percentile. These four classrooms also had the highest number of study participants,

indicating that the higher the participation per classroom, the greater the decrease in BMI.

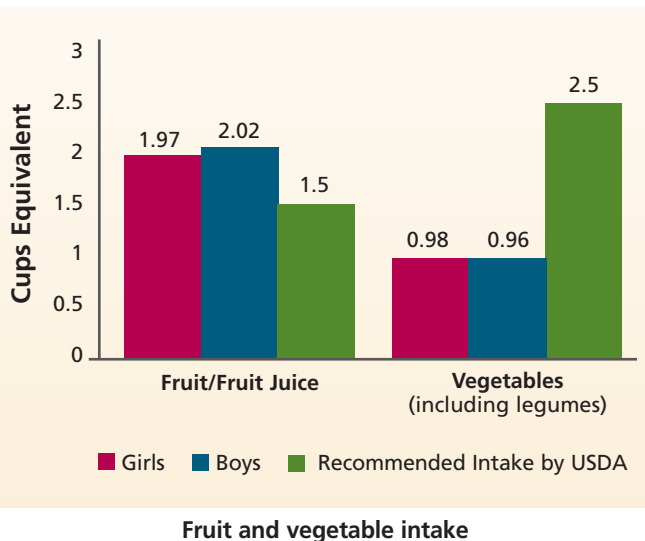
Interestingly, we observed a similar trend in BMI change among the non-study children in all 3<sup>rd</sup> grade classrooms. These results probably reflect teacher support for the study. It is likely that support by these teachers had an impact on the health behavior of their students regardless of the students' participation in the study.

	Fall 2009	Spring 2010
<b>Number of Children Overweight or Obese</b> (≥85th Percentile)	<b>21 (28%)</b>	<b>17 (23%)</b>
<b>Number of Children with Healthy Weight</b> (<85th Percentile)	<b>54 (72%)</b>	<b>58 (77%)</b>

**BMI at the beginning and end of the study**  
(Number of children = 75 for whom we had complete BMI data)

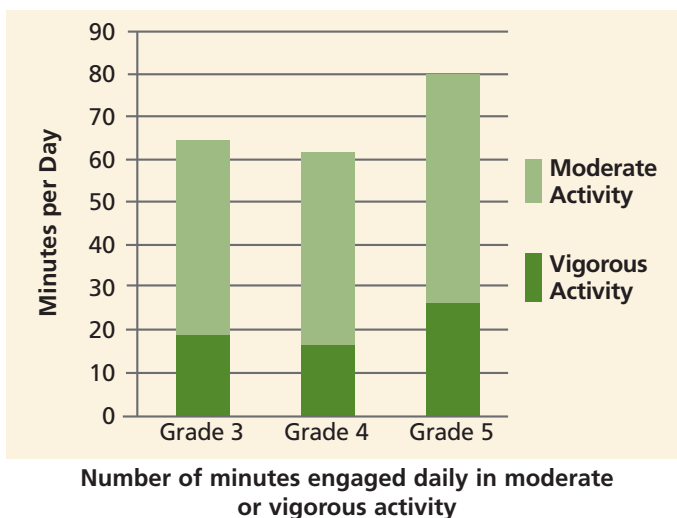
### Fruit/Fruit Juice and Vegetable Intake

The Food Questionnaires revealed that the fruit/fruit juice and vegetable intake did not differ between girls and boys (see figure below). The children consumed about half a cup more fruit than recommended by the U.S. Department of Agriculture. However, reported vegetable consumption was significantly below the recommended intake of 2.5 cups. The increased fruit intake is likely due to the fresh fruit offered for morning snack. The reported fruit and vegetable intake did not change significantly from the beginning to the end of the study. The Food Questionnaires also revealed that most children did not get the recommended amounts of vitamins A and E, calcium, and magnesium from their diet, and many also did not get enough vitamin K, iron, and zinc. We were unable to assess vitamin D status because we could not obtain blood samples from the children.



## Moderate and Vigorous Physical Activity

The Physical Activity Survey showed that the mean daily physical activity time in all three grades was 60 minutes or more (see figure below) and did not change significantly throughout the study, although the participation in walking/running declined, as mentioned above. These data indicate that the children met the recommended guidelines of 60 minutes of moderate to vigorous physical activity daily but may also suggest that the students didn't report their level of activity accurately.



## Conclusions

Our study was successful in recruiting more than 50% of eligible students, especially females, and kept a remarkable 75% of enrolled students motivated throughout the entire school year to improve their eating and physical activity habits.

The high level of participation, the decrease in BMI, and the low drop-out rates in the three 3<sup>rd</sup> grade classrooms and one 5<sup>th</sup> grade classroom suggest that teacher support is a critical influence on the students' health behaviors, a concept that needs to be further analyzed and confirmed. It is also likely that parent support and peer pressure influence children's health behaviors.

The study also indicated that children are more likely to eat fruits and vegetables when given the opportunity and encouragement. In contrast, study participation in physical activity was low among 4<sup>th</sup> and 5<sup>th</sup> graders and declined over time in all grades, suggesting that it needs to be more encouraged and incentivized, especially given the key role of physical activity in maintaining a healthy weight.

The *LPI Fitness & Nutrition Study* was supported by grants from the Spirit Mountain Community Fund and the Juan Young Trust, both in Oregon, and a generous gift from an anonymous donor. The children's multivitamin/mineral supplement was a gift from USANA Health Sciences Inc., Salt Lake City, UT. **LPI**

## Annual Conference of the Oxygen Club of California

Stephen Lawson, LPI Administrative Officer



The Oxygen Club of California held its biennial scientific conference—co-sponsored by LPI—in Santa Barbara, California, in mid-March. *Oxidants and Antioxidants in Biology: Translational Redox Science* featured 44 presentations in six sessions: *Wound Healing*; *Redox Signaling and Inflammation*; *Cardiovascular*; *Translational Science by Micronutrients*, which included subsessions on *Carotenoids and Vitamin A*, *Coenzyme Q*, and *Polyphenols*; *Redox Imaging*; and *Aging*. There was also a poster session with over 100 presentations covering an extraordinary range of topics.

The keynote lectures were given by two of the 2008 Nobel Laureates in Physiology or Medicine, Harald zur Hausen and Luc Montagnier. Zur Hausen won “for his discovery of human papilloma viruses causing cervical cancer” and Montagnier won, together with Françoise Barré-Sinoussi, “for their discovery of human immunodeficiency virus.” In his presentation on “Novel infectious agents in human carcinogenesis: State and perspectives,” zur Hausen discussed the potential effect of vaccinations on preventing cancers caused by hepatitis B virus and human papillomaviruses. He addressed the mechanism by which certain viruses can cause the transformation of normal cells to malignant cells, as well as the combined effects of viral

infections, dietary factors, and other influences in that process. Montagnier talked about “New horizons in HIV/AIDS,” including his development of novel technology to detect persistent reservoirs of HIV DNA in the blood of patients undergoing combined antiretroviral drug therapy.

Tissue injury triggers a number of responses that aid in healing. Speakers in the first session addressed this multifactorial response, which includes recruitment of repair signals and cells to the injury site stimulated by either hypoxia (reduced oxygen tension) in bone marrow or hyperbaric oxygen treatment, which also stimulates angiogenesis (new blood vessel formation) at the wound.

Speakers in the second session focused on redox (reduction “electron gain”—oxidation “electron loss”) signaling and regulation, which play important roles in cellular metabolism, immune function, response to injury, inflammation, and cell growth. The balance between oxidants and endogenous antioxidants like thioredoxin, glutaredoxin, and peroxiredoxins controls, among other things, the fate of cells. In rats, resveratrol was shown to improve cardiac parameters, including stem cell performance, after coronary artery occlusion. In old rats, R-alpha lipoic acid improved antioxidant capacity in the liver not by acting itself as an antioxidant but rather by reversing age-related



declines in the endogenous antioxidant glutathione. Redox imaging was discussed in the fifth session, with presentations on new instrumentation and molecular probes that provide imaging and measurements of reactive oxygen species *in vivo*.

In the third session on cardiovascular diseases, speakers explained how redox status affects diabetes-related impairment of angiogenesis after myocardial infarction in rats. Treatment with a virus containing the gene for the antioxidant thioredoxin improved outcomes. Thioredoxin also exhibits cardioprotective effects related to the growth and death of heart muscle cells. Oxysterols, the oxidized metabolites of cholesterol found in food and produced in the body, are associated with the development of atherosclerosis and could, because of their inflammatory potential, contribute to other diseases.

The Norman I. Krinsky Memorial Lecture focused on the metabolism of carotenoids. Norman Krinsky, who made seminal contributions to carotenoid research during a long and productive career at Tufts University, died in 2008. Carotenoids are colored compounds, some of which produce vitamin A and play important roles in eye health and immunity. Several speakers discussed genetic polymorphisms (variations) that probably account for the great variability among humans in carotenoid absorption and conversion. Indeed, intake of beta-carotene, which is converted into vitamin A, does not always predict vitamin A status. Vitamin A may also have an important effect on energy production in the mitochondria.

Coenzyme Q, which also plays an important role in the production of chemical energy in the mitochondria, may affect apoptosis (programmed cell death) and aging. Coenzyme Q reduces the age-related expression of oxidase enzymes that generate superoxide radicals, which are damaging reactive oxygen species. Treatment of astrocytes (glial cells in the central nervous system) in culture or human subjects with statins—drugs administered to lower cholesterol levels—substantially decreased coenzyme Q levels.

Inflammatory pathways that figure in chronic diseases may be modulated by certain dietary factors, including resveratrol and curcumin from the spice turmeric, as well as a host of other spices. In mice, resveratrol also shares similarities with caloric restriction in retarding aging (represented by gene analysis), especially in the heart.

Polyphenols from plants affect redox pathways, thereby increasing antioxidant and detoxification capacity. However, polyphenols like ellagitannins from pomegranates have very limited bioavailability—only very small amounts are absorbed into the blood stream from the gut—and are extensively and rapidly chemically modified after absorption. Therefore, it is difficult to extrapolate from cell culture studies to *in vivo* behavior and from parent compounds to their metabolic derivatives.

The last session focused on aging. Researchers showed that specially bred mice lacking the *RasGrf1* gene involved in cognitive function and glucose homeostasis had an increased lifespan that mimicked the effect on lifespan by caloric restriction. Another speaker discussed the need for oxidized protein to be degraded and removed from cells; otherwise, protein aggregates can form that result in cellular senescence.

Age-related dysfunction in vascular cells (the endothelium) caused by oxidative stress and inflammation is similar to endothelial changes observed in young people with diabetes or hypertension. The “triage theory” was advanced by Bruce Ames to explain how micronutrient deficiencies throughout evolution result in metabolic rebalancing to favor those substances required for short-term survival while starving those needed for long-term health. Chronic micronutrient deficiencies, which are prevalent even in apparently well-nourished societies, may contribute to the incidence of age-related disease.

Bharat Aggarwal of the M. D. Anderson Cancer Center of the University of Texas won the \$25,000 Oxygen Club of California Jarrow Formulas Health Sciences Prize for his long-term work on the role of natural products, especially spices, in attenuating chronic inflammation associated with the development of cancer.

Based on their poster presentations, Jennifer Ehren of The Salk Institute for Biological Sciences and Ryan Hamilton of the University of Southern California won LPI Young Investigator Awards. Dr. Ehren showed that fisetin, a flavonoid in strawberries and other fruits, improved multiple physiological parameters, including nerve and kidney function, when fed to diabetic mice. Dr. Hamilton discussed findings showing that caloric restriction when young may help prevent hypometabolism and mitochondrial dysfunction associated with Alzheimer’s disease when older. **LPI**



## FACULTY HONORS

LPI’s Maret Traber was elected President of the Oxygen Club of California (OCC) for 2010-2012, succeeding Chandan Sen of Ohio State University. LPI and OCC

co-sponsor scientific meetings and have enjoyed a long and productive association because of a shared interest in the roles of reactive oxygen species and antioxidants in health and disease.

LPI Director Balz Frei gave the 2010 *Denham Harman, M.D., Ph.D., Lecture in Biomedical Gerontology* at the University of Nebraska Medical Center in Omaha, Nebraska, on April 9, 2010. Dr. Harman proposed the seminal and highly influential “free radical theory of aging” in 1956, followed in 1972 by the hypothesis that mitochondria in cells may act as a biological clock by producing free radicals that damage organelles and biomolecules, leading to senescence. Dr. Frei’s lecture was entitled “Oxidative Stress and Inflammation in Aging and Atherosclerosis: Ameliorating Effects of Lipoic Acid.”



Balz Frei (left) and Denham Harman (right)



## Nutrition and Immunity, Part 2

Victoria J. Drake, Ph.D.  
LPI Research Associate  
Manager, Micronutrient Information Center

*Summary: Deficiencies of zinc, selenium, iron, and copper adversely affect immune response. Conversely, too much iron and copper impair immune function. Probiotics—microorganisms often added to dairy products like yogurt—improve immune function in the gastrointestinal system and may help prevent inflammatory bowel disease. Obesity produces chronic inflammation and compromised immunity that may increase the susceptibility to infections. Moderate, regular exercise may enhance immunity, but prolonged high-intensity exercise may impair it.*

Part 1 of this article (LPI Research Newsletter Spring/Summer 2010) discussed the differences between the innate and adaptive immune systems and focused on the role of macronutrients (protein and lipids) and vitamins. In part 2, I discuss the role of minerals and other dietary and lifestyle factors in immunity.

Several nutritionally-essential minerals, including zinc, selenium, iron, and copper, play important roles in the development and expression of immune responses. Zinc is required for both innate and adaptive immunity because it has various catalytic, structural, and regulatory functions in the body. Inadequate intake of zinc can lead to a nutritional deficiency of the mineral and compromised immune function. For instance, zinc deficiency impairs the complement system, a biochemical network of more than 30 proteins in plasma and on cell surfaces that functions to kill invading pathogens by direct lysis (cell rupture) or through the promotion of phagocytosis. Phagocytosis is a process by which certain immune cells, such as macrophages, engulf and digest invading microorganisms and foreign particles. Zinc deficiency also impairs other components of innate immunity, including natural killer cell activity and the ability of immune cells to generate oxidants that kill invading pathogens, as well as production and function of lymphocytes—cells that are key to mounting an adaptive response. Adaptive immune responses, which are more complex than innate responses, provide antigen specificity and immunologic “memory” of pathogens; the latter makes subsequent responses to the same pathogen more efficient. For example, vaccines function by this process so that subsequent exposure to the pathogen elicits a fast and efficient immune response. Zinc deficiency results in a heightened vulnerability to several infectious agents. In particular, children with zinc deficiency have increased susceptibility of infectious diarrhea, and zinc supplementation reduces the frequency, severity, and duration of diarrheal episodes in young children. Zinc supplementation in children may also reduce the incidence of lower respiratory infections like pneumonia. However, because of conflicting studies, it is presently not clear whether zinc supplementation has utility in treating childhood malaria.

Selenium is required for normal function of several enzymes important in innate and adaptive immunity, including the glutathione peroxidases—key redox regulators and cellular antioxidants. Inadequate intake of selenium can impair multiple immune responses, such as cytokine (cell-signaling molecules) expression, antibody production, and aspects of cell-mediated immunity. Selenium deficiency has been shown to enhance the virulence or progression of some viral infections. For example, mouse studies have shown that a relatively harmless strain of coxsackievirus becomes more virulent in selenium-deficient mice, resulting in an inflammation of the heart muscle called myocarditis. In humans, concomitant selenium deficiency and coxsackievirus infection may both contribute to the cardiomyopathy in Keshan disease. Additionally, small controlled trials in individuals who were not overtly selenium deficient have found that short-term supplementation with selenium enhances immune cell response to foreign antigens. Other studies have established selenium to be an important regulator of cytokine expression.

Iron is required by the host to mount an effective immune response because the mineral is needed in the differentiation and proliferation of T lymphocytes and in the generation of reactive oxygen species that kill pathogens. Accordingly, iron deficiency—the most prevalent micronutrient deficiency in the world—results in impaired immunity and increased morbidity and mortality from infections. However, iron is required by most infectious agents for replication and survival, and during the early stages of an infection, serum levels of iron decrease and ferritin (a protein that stores iron) levels increase in order to sequester iron from pathogens. Moreover, elevated iron levels, such as in untreated hereditary hemochromatosis (a genetic condition of iron overload despite normal iron intake), can impair phagocytic function, cytokine production, complement system activation, as well as the function of B and T lymphocytes. Further, elevated iron levels may be a risk factor for cancer and death, especially in men. Since iron is very efficiently recycled in the body and lost only in blood and skin sloughing, the Linus Pauling Institute recommends that men and postmenopausal women who are not at risk of iron deficiency take a multivitamin-mineral supplement that does not contain iron.

Copper is also important in immunity, but the exact mechanism of its immune action is not yet known. Nutritional deficiency of copper results in an abnormally low number of neutrophils, a condition called neutropenia. Menkes disease is a genetic disorder of intracellular copper transport. Individuals with Menkes disease suffer from severe copper deficiency and frequent, serious infections. It is currently unknown if marginal or mild copper deficiency results in impaired immunity, but high intakes of copper for prolonged periods have been shown to adversely affect immune function.

Dietary factors other than nutrients may affect immunity, as well. For instance, yogurt and other fermented foods may contain probiotics, which are live microorganisms that benefit the overall health of the host when they are administered in sufficient amounts. Bacteria of the





*Lactobacilli* and *Bifidobacteria* species are common examples. These and other probiotics can transiently inhabit the lower gastrointestinal tract and modulate immune function by interacting with intestinal epithelial cells and immune cells. Studies have shown that probiotics can benefit both innate and adaptive immunity; however, immune modulation requires regular consumption of probiotics since they have not been shown to permanently alter intestinal microflora. Specific immune effects include strengthening the intestinal epithelial barrier and stimulating production of antibodies and T lymphocytes—important mediators of the adaptive immune response. Immune effects of probiotics may depend on the particular strain, as well as the dose, route, and frequency of delivery. While probiotics may have utility in the prevention of various diseases, such as inflammatory bowel disease, diarrheal diseases, allergic diseases, and gastrointestinal infections, more clinical studies are currently needed to elucidate their health effects.

While certain nutritional deficiencies, like some of the mineral deficiencies mentioned above, can compromise immunity, oversupply of nutrients may also be associated with untoward immune effects. Overnutrition is a form of malnutrition where nutrients, especially macronutrients, are supplied in excess of the body's needs. Overnutrition can create an imbalance between energy intake and energy expenditure, leading to excessive energy storage and obesity. Obesity is a major public health problem in the United States and elsewhere because the condition is associated with increased risk of morbidity from a number of chronic diseases, including hypertension and other cardiovascular diseases, type 2 diabetes, liver and gallbladder disease, osteoarthritis, sleep apnea, and various cancers. Moreover, obesity is linked to an increased risk of overall mortality.

Obesity has been shown to alter immunocompetence. In obesity, immune cells called macrophages infiltrate adipose (fat) tissue and accumulate in numbers proportional to the degree of obesity. Macrophages and other immune cells play important roles in the development of inflammation. Inflammatory processes are triggered in part by molecules secreted by adipose tissue. A chronic state of low-grade inflammation exists in obesity. Adipose tissue secretes a number of fatty acids, cytokines, and hormones that are involved in inflammatory and immune processes. The hormone leptin is secreted from adipose tissue and circulates in direct proportion to the degree of fat stores. Leptin is an important regulator of food intake, body

weight, and energy homeostasis. Results of animal and *in vitro* studies indicate that leptin also modulates inflammatory and other immune responses, such as stimulation of pro-inflammatory cytokine production. Immune modulation that occurs in obesity could increase the susceptibility of obese individuals to infections. Some epidemiological studies have shown that obese patients have a higher incidence of postoperative and other hospital-related infections compared with patients of normal weight. Obese individuals have also been shown to exhibit poor wound healing and increased occurrence of skin infections.

An increased vulnerability, severity, or complications of certain infections in obesity may be related to a number of factors, such as select micronutrient deficiencies. In fact, deficiencies or inadequacies of the B vitamins and vitamins A, C, D, and E have been associated with obesity, probably because energy-dense but micronutrient-poor foods are consumed. Certain mineral deficiencies may also be linked to obesity. For example, one study in obese children and adolescents associated impairments in cell-mediated immunity with deficiencies in zinc and iron. Overall, immune responses appear to be compromised in obesity, but more research is needed to clarify the relationship between obesity and infection-related morbidity and mortality.

In addition to dietary factors, lifestyle choices may play a role in immunity. Moderate, regular physical activity decreases biomarkers of systemic inflammation and may also enhance immune function, especially in sedentary individuals. In contrast, high-intensity exercise for prolonged periods ( $\geq 90$  minutes) increases levels of C-reactive protein—a biomarker of cardiovascular and systemic inflammation—and may temporarily compromise responses of both innate and adaptive immunity. However, the effects of exercise on immune responses are probably influenced by a number of variables, including a person's age, genetics, overall health and nutritional status, as well as the type, intensity, and duration of exercise. More clinical research is needed to determine whether exercise-induced changes in immune functions translate to altered risk of various infections, such as the common cold and other respiratory tract infections.

For more detailed information on nutrition and immunity, see the article in the Micronutrient Information Center: <http://lpi.oregonstate.edu/infocenter/immunity.html>. This article was underwritten, in part, by a grant from Bayer Consumer Care AG, Basel, Switzerland. **LPI**

### Kudos for the LPI Web site

"Thank you for making objective, scientifically-rooted nutritional information available on your website. Since the internet is littered with unsubstantiated, bizarre, and sometimes dangerous nutritional claims and advice, a site that offers well-organized, clearly stated empirical information is invaluable. You are without peer in this regard, so I always turn to your site first when I need information. Please keep up the good work!"

— Dr. Jim Conner, September 2010



# Linus Pauling Institute

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## Micronutrient Research for Optimum Health



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### The Linus Pauling Science Center under construction

To see the progress on our building, please visit our real-time Web cam at <http://webcam.oregonstate.edu/lpsc>.

LPSC is scheduled for completion in summer 2011.



## Look for these informative articles inside!

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