

# The Linus Pauling Institute

RESEARCH NEWSLETTER



## From the Director

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

Planning for the new \$62.5-million Linus Pauling Science Center (LPSC) at Oregon State University is in full swing. As part of the programming phase for the building, we have had three two-day meetings over the last three months with our architect firm, Zimmer Gunsul Frasca LLC; laboratory design firm, Research Facilities Design; and general contractor, Andersen Construction Company. The principal results of this programming phase are that the building will house the Linus Pauling Institute, part of OSU's Department of Chemistry, including research and teaching laboratories, an instrument suite with Nuclear Magnetic Resonance (NMR) and electron microscopy equipment, a vivarium, and a state-of-the-art auditorium for teaching and conferences. We are now entering the schematic design phase, which will be followed by the design development phase and, eventually, the preparation of the detailed construction documents. These documents should be completed by early next year, allowing us to break ground for the building in the spring of 2009.

The Linus Pauling Institute will occupy at least one floor of LPSC, which will provide space for about 12 research laboratories, laboratory support facilities, a conference room and library, and offices and other support spaces for about 90 researchers and administrative staff. We currently have 10 research laboratories in the Institute spread over three buildings of the OSU campus and a total of 70 researchers and four administrative staff. The current plan is to move all but one of the existing research laboratories into the new building upon its completion, projected for late 2010. This will give us enough space in LPSC to expand by three research laboratories. As I mentioned in the last Research Newsletter,

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## Cancer Chemoprotection

*An interview with George Bailey, Ph.D.  
Distinguished Professor Emeritus  
LPI Principal Investigator Emeritus*

**Q. How did you get interested in cancer chemoprotection?**

**A.** When I first came to Oregon State University in 1978 on sabbatical leave, I was interested in studying the structure of chromosomes with Ken van Holde. The nucleosome structure hadn't been worked out at all, and during the course of my year I read about work on the interaction of carcinogens with chromatin. Nucleosomes are structures in the cell's nucleus that package DNA into chromosomes, and chromatin is made up of DNA and histone proteins that together form chromosomes. So I switched into that area and looked at the interaction of the carcinogen aflatoxin with nucleosomes from steelhead trout that I caught in the Alsea River. Eventually I accepted an offer to join Russ Sinnhuber's group in Food Science at OSU. They had a contract through the National Cancer Institute to use rainbow trout to test a few chemical compounds, most of them food derived, that had just been discovered as having cancer chemoprotective activity. We found that one such compound, indole-3-carbinol from cruciferous vegetables, was very effective. Then we became interested in identifying molecular mechanisms and determining dose-response relationships.

**Q. Why use trout as a model of carcinogenesis instead of small rodents?**

**A.** Well, I like trout, and they're interesting from an evolutionary perspective, too. Trout are extremely low cost to purchase and to house. That allows us to use thousands of trout in an experiment to achieve high statistical power in examining dose-response issues. To do that with rats or mice would be too costly.

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— DESIGNATED BY THE NATIONAL INSTITUTES OF HEALTH AS A —

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

we are planning to use these new laboratories to establish and expand the Healthy Aging Program in LPI. The goal of this program is to better understand the underlying molecular and biological causes of aging and how diet and dietary factors can favorably influence the aging process and reduce the risk of age-related chronic diseases.

We have already started our search to fill the first position, which will be in the area of “epigenetics” in aging or immunosenescence. Epigenetics is the study of heritable changes in gene function that occur without a change in the DNA sequence, leading to differential gene expression—genes being turned “on” or “off,” which can accelerate or delay aging and chronic disease. Immunosenescence refers to the causes of the age-related decline in immune function and how this can be slowed by diet and supplements. We have identified some outstanding candidates for this position and hope to fill it later this year. The other two

positions will be in the molecular biology of aging and in age-related neurodegenerative diseases, such as Parkinson’s or Alzheimer’s disease.

The future has never looked brighter for LPI, and I am excited and grateful to be part of this transformation and growth of the Institute. The new knowledge emanating from the Healthy Aging Program and the other research laboratories in the Institute on the biology of aging and the prevention of cancer, cardiovascular and metabolic diseases, and neurodegenerative diseases will allow us to even better serve and advise the public and health-care professionals about a healthy diet, the right supplements, and a healthy lifestyle. It is only befitting that this research will be conducted in a building dedicated to one of the greatest scientists of the 20<sup>th</sup> century and the founder of our Institute, Dr. Linus Pauling. **LPI**

## Inauguration of the Burgess and Elizabeth Jamieson Chair in Healthspan Research



A special event at Oregon State University on April 22<sup>nd</sup> celebrated the new endowed Burgess and Elizabeth Jamieson Chair in Healthspan Research in LPI. Dr. Tory Hagen, director of LPI’s Healthy Aging Program, will hold this Chair. Dr. Hagen joined LPI in 1998 after finishing a post-doctoral fellowship in the laboratory of Dr. Bruce Ames at the University of California-Berkeley. Dr. Ames, a member of the National Academy of Sciences, is a recipient of the National Medal Of Science and the LPI Prize for Health Research. Prior to joining Dr. Ames’s lab, Dr. Hagen received his Ph.D. in biochemistry from Emory University in Atlanta. He is an LPI Principal Investigator and an associate professor in OSU’s Department of Biochemistry and Biophysics.

LPI Director Balz Frei, OSU Foundation President Mike Goodwin, and OSU President Ed Ray thanked the Jamiesons for their transformational gift. Ed Ray saluted Burgess Jamieson as a venture capitalist and co-founder of Sigma Partners in California who “shared two beliefs that Linus Pauling certainly would have held: pursue great ideas and invest in creative, productive people who can translate these ideas into realities.”

Dr. Hagen worked on glutathione, an important biological antioxidant, in Dr. Dean Jones’s lab at Emory. He published papers on the bioavailability of glutathione and its protection of tissues from oxidative damage, and started to think about oxidative stress in mitochondria, the organelles in cells that produce chemical energy, and

aging. In the Ames lab, Dr. Hagen worked on age-related mitochondrial dysfunction and its remediation in old rats with the dietary supplements acetyl-L-carnitine and alpha-lipoic acid. Drs. Hagen and Ames found that supplementation with acetyl-L-carnitine, a nonprotein amino acid that shuttles fatty acids into the mitochondria where they are burned to generate energy, increased physical activity and improved cognitive function in old rats by restoring mitochondrial activity. They also found that co-supplementation with alpha-lipoic acid, a mitochondrial antioxidant, further improved performance. Additionally, alpha-lipoic acid normalized vitamin C status in old rats, which was found to decline with age.

Dr. Hagen and his colleagues have more recently been investigating the age-related decline in the efficiency of a cellular vitamin C transporter, as well as the roles of alpha-lipoic acid in cell signaling apart from its antioxidant function, in protecting the liver from toxic insults, and in cardiac, brain, and endothelial function.

The Healthy Aging Program was recently established at LPI to help everyone achieve their maximum healthspan through optimum health. In the expansion of this initiative, LPI will recruit three new faculty to explore and better understand the biological causes of aging and how they are affected by diet, micronutrients, and lifestyle.

The Jamieson’s extraordinary gift will allow LPI to accelerate the development of our Healthy Aging Program, and we are grateful for their generous support to create the Jamieson Chair. **LPI**

**Q. Does that allow you to make observations on dose-response relationships at low doses of carcinogens?**

A. Absolutely! I'll give you an example—the simple question of assessing cancer risk at a high dose and extrapolating that to low doses and low tumor incidences relevant to humans. All the data available for risk assessment come from rodent experiments with very high carcinogen exposures, allowing tumor incidence in the 10-100% range. You can measure that incidence with a reasonable number of animals. But we want to know what dose might give one extra cancer per one million individuals because that's the regulatory target we're reaching for. Unfortunately, that requires extrapolating the high dose data 100,000-fold. Using rainbow trout, we conducted the largest ever dose-response study in any animal model to get information on low doses of carcinogens that are relevant to human exposure. The default assumption had been that the risk is linear below 10% tumor incidence, but in our experiment with 42,000 trout, we found that assumption to be false. We found, instead, a threshold.

**Q. Are trout studies relevant biologically to humans?**

A. Yes. There's tremendous evolutionary conservatism in biology. For instance, patterns of gene expression and altered gene expression are very similar in liver cancer in both humans and trout. We share with trout a similar set of enzymes to metabolize carcinogens. Trout don't repair DNA damage as well as humans do—they are a bit more sensitive—but that doesn't change the shape of the dose-response curve.



**Q. Do you compare responses in trout to rodents?**

A. We are looking at the inhibition of cancer by chlorophyll and its derivative, chlorophyllin, using two different carcinogens in rainbow trout. We extrapolate that to rats. With LPI Principal Investigator Dave Williams' transplacental mouse model and Rod Dashwood's rat colon carcinogenesis model, we then looked at whether the cancer chemoprotective mechanisms are similar in rodents and people. So we don't focus exclusively on trout.

**Q. Do trout require a special facility?**

A. Virtually all the money for our facility and its expansion and improvement came from NIH grants to Russ Sinnhuber, Jerry Hendricks, Dave Williams, and me. The National Institutes of Health has encouraged investigators to develop alternative models for human health research. I served as director of the Marine and

Freshwater Biomedical Sciences Center at OSU for 17 years. The center was sustained by about \$8 million in grants from the National Institute of Environmental Health Sciences. That support was absolutely critical in developing trout as a model for human carcinogenesis and chemoprotection. Of course, access to a good, clean water supply is critical. Some aquarium fish models, like zebra fish, have become popular because their housing requirements are somewhat easier to meet. Our trout facility at OSU is probably the only one in the world that permits us to do our kind of research.

**Q. You mentioned studies with chlorophyll and chlorophyllin in trout. What's the difference between chlorophyll and chlorophyllin?**

A. Chlorophyllin is a derivative of chlorophyll, the natural green pigment in plants. It's obtained from natural chlorophyll through a hydrolysis process that converts fat-soluble chlorophyllin into a water-soluble dye that can be used commercially, for instance, as a food coloring agent and deodorant. There's a long history of chlorophyllin use in the health care industry.

**Q. Why use chlorophyllin instead of natural chlorophyll?**

A. Natural chlorophyll is very unstable, and it deteriorates very quickly after isolation and purification. With the chlorophyllin derivative, through hydrolysis and replacement of magnesium—the metal ion that's naturally found in chlorophyll—with copper, you get a very stable compound that doesn't oxidize or degrade as quickly as chlorophyll. You might think that replacing magnesium with copper would make the compounds more reactive, but the copper is very tightly bound, so it's not available to engage in detrimental reactions.

**Q. Have you compared the difference in chemoprotection between the purified compound and the food in which it's found?**

A. We can't do that with fish because they are carnivores, not herbivores. They don't digest plant material. Our human studies are all done with purified chlorophyll or chlorophyllin, not with spinach, which is rich in chlorophyll. So that experiment remains to be done. One study that we recently completed in Rod Dashwood's lab used freeze-dried spinach in rats. He got an interesting result in that experiment. When the spinach was fed after colon cancer was initiated in the rats, it had a protective effect against carcinogenesis. It was pretty impressive, and we didn't expect it. We expected to see a protective effect when the spinach was given at the same time as the carcinogen, but that was not observed. So we have to sort it out. Is it because the chlorophyll isn't bioavailable? Was it given appropriately with the carcinogen dose? There are a lot of variables to examine when you get a surprising finding like that.

**Q. Are there any side effects or toxicity with either chlorophyll or chlorophyllin?**

A. Nobody is aware of any toxicity issues for natural chlorophyll. The FDA allows a level of intake of

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chlorophyllin that is in the range of 100-150 milligrams per tablet. No toxicity has ever been reported, and we haven't observed any in our short-term studies with humans. In future long-term human trials, there is a potential risk of tumor promotion by chlorophyllin based on the rat colon carcinogenesis model. So, under certain circumstances, at certain doses of chlorophyllin and exposure to certain carcinogens, there may be some risk. Interestingly, we observed protection in both very low and very high doses. In those experiments, chlorophyllin was given after cancer initiation—after the carcinogen was administered.

**Q. You've also studied indole-3-carbinol from cruciferous vegetables as a cancer chemoprotective agent. Is it safe and effective?**

A. Indole-3-carbinol, or I3C, is the classic example of the double-edged sword in cancer chemoprotection. We were the first to observe this in the early 1980s. When we did our first experiments with I3C, we gave it with the carcinogen aflatoxin to trout and with a different carcinogen, dimethylbenzanthracene, to rodents. I3C helps to block the cancer process when it's given at the same time as the carcinogen. Also, I3C was given to rats in breast cancer experiments because of its anti-estrogen effects. In that model, I3C suppressed mammary tumor development. So there has been tremendous interest in the compound for that reason.

In our experiments with trout, we asked if I3C would protect against liver cancer if given over a protracted period. Unfortunately, it promoted liver cancer. Years later we continued that work with rats and saw the same effect. Oddly enough, I3C shows this tumor promotional property because in the liver itself it's estrogenic. The public and the scientific community get very confused by this. It's one of the first examples of a compound that may reduce the risk of prostate cancer or breast cancer in people who are at very high risk of these cancers, but that may be offset by some increased risk for liver cancer. It's a difficult risk-benefit equation.

**Q. Does that suggest that people should perhaps avoid I3C supplements and instead eat a good amount of cruciferous vegetables?**

A. I would not take I3C supplements, nor would I recommend them. We don't know enough about mechanisms and nuances to gauge risk versus benefit in this situation.

**Q. Some years ago you and colleagues at Johns Hopkins were involved in a clinical trial in China to assess the cancer chemoprotective effects of supplemental chlorophyllin. What did you find?**

A. This is the study that I felt took the rainbow trout model full cycle for the first time. Liver cancer was discovered in rainbow trout in fish hatcheries in the Pacific Northwest in the early 1960s. That was traced to aflatoxin, a mold toxin that contaminated the cottonseed oil used for part of the fish diet. Building on evidence from rats and then turkeys, it became clear that aflatoxin is an important risk factor for liver cancer in humans. We now know that aflatoxin exposure in certain parts of the world is one of the greatest risk factors for liver cancer, along with hepatitis virus infection. These two risk factors synergize, such that liver cancer is one of the leading causes of cancer death world-wide. We need remediation because exposure to aflatoxin in food in some areas is unavoidable. You can't always see the contamination in foods. There's a vaccination program for protecting against viral hepatitis risk, but that's expensive and not easily available to people at risk in third-world developing countries. Since those people have an unavoidable dietary exposure, we need to find strategies to mitigate cancer risk. There are compounds that occur naturally in foods,

as well as some drugs, that will help to block liver cancer in animals exposed to aflatoxin. They do this by increasing the expression of enzymes important in aflatoxin metabolism.

In trout we discovered that chlorophyllin worked against aflatoxin very effectively by a much simpler mechanism—binding to the aflatoxin so that it does not get absorbed as efficiently into the body. The absorption of the carcinogen is reduced, and more is excreted in the feces. That biophysical

process should be independent of species. Those results led to the intervention trial in China where there are people unavoidably exposed to aflatoxin in their diet, especially from contaminated corn and rice. Colleagues at Johns Hopkins and I enrolled 180 people in that trial. Half of them—90 people—took a placebo tablet with each meal for three months. The other 90 people took a 100 milligram tablet of chlorophyllin with each meal for three months. Then we tested their effective aflatoxin uptake. We would have to follow them for over 20 years to observe liver cancer, so we instead measured a cancer biomarker—how much aflatoxin DNA repair products appeared in their urine over a 24-hour period.

Chlorophyllin should be effective in reducing the aflatoxin uptake in the supplemented people. Less aflatoxin should get to their livers, less should get activated, less should bind with DNA, and less should get excreted in urine. This is indeed what we found—the people who received the chlorophyllin tablet had a 55% decrease in cancer biomarkers compared to those who received the placebo. So the trout model played an important role in helping to discover a method to attenuate aflatoxin-induced carcinogenesis. It played a role in helping to discover an effective chemoprotective agent that costs pennies a day anywhere around the world.

**Q. Are those results persuasive enough to recommend that people unavoidably exposed to dietary aflatoxin should take chlorophyllin supplements? Or do you need further clinical trials?**

**A.** There is absolutely no reason that definitive clinical trials shouldn't be done now. The people in China know how to do it, they're equipped to do it, and they don't need anybody else to guide them. It's up to them. In affected areas in China, about 10% of men die from liver cancer by the age of 45. That's a huge death rate from a single cancer, and I believe that could be halved for pennies a day.

**Q. Does the Chinese government show much interest in promoting this kind of clinical work?**

**A.** The Chinese government has cooperated a lot in making this population available for these kinds of studies by foreign scientists. And I think they will continue to do that.

**Q. Is aflatoxin exposure a concern for people living in the United States?**

**A.** Not in the United States, because we're wealthy enough to afford the Food and Drug Administration and USDA that test our food supply for aflatoxin contamination. The nuts, grains, and seeds that are normally contaminated elsewhere, especially in the developing countries, are tested, the food supply is screened, and the action level is to exclude more than 15 parts per billion of aflatoxin in any food—peanut butter, corn, and other grains.

**Q. So we don't need to worry about corn, peanut butter, or roasted peanuts?**

**A.** Not in this country. But in Africa, which is another hot spot for liver cancer, people have high rates of hepatitis viral infection coupled with high rates of aflatoxin exposure from peanuts.

**Q. How does indole-3-carbinol protect against liver cancer caused by aflatoxin? Is it a mechanism different from the blocking mechanism you described with chlorophyllin?**

**A.** Yes, it is. There are several possible mechanisms, depending on the animal models you examine. One mechanism involves inducing enzymes in the liver that detoxify aflatoxin. These can be the cytochrome p450s that metabolize aflatoxin B1 into a form that is ten-fold less carcinogenic. We see that in dairy cows and trout. A second protective mechanism is the induction of glutathione transferase isozymes that detoxify the aflatoxin before it can damage DNA. That mechanism does not exist in the trout, but it does exist in the rat and is thought to be important in humans. A third mechanism that we discovered in the trout gets rather little attention, but it's probably more important than most of us think. It involves the binding of I3C metabolites to enzymes that are necessary to activate the carcinogen. With rainbow trout it's easy to show that physiological levels of I3C severely inhibit the activity of the enzyme that converts aflatoxin to its carcinogenic form. That mechanism has been shown to operate in all species that have been examined.

**Q. Over twenty years ago you published some studies using a synthetic flavonoid called beta-naphthaflavone and the preservative BHA in preventing liver carcinogenesis. You also investigated nitrosamines. What did you find?**

**A.** Beta-naphthaflavone was just as potent as I3C dose for dose. It's a compound that acts in the trout by the mechanisms I mentioned—it's an inhibitor of the enzyme that activates aflatoxin. In rodents, it induces phase 1 and phase 2 detoxification enzymes. It doesn't induce phase 2 enzymes in trout though. BHA has been shown to be generally quite effective as a cancer chemoprotective compound in rodents. We did some studies in collaboration with Dick Scanlan in the Food Science Department at OSU. We tested I3C in rainbow trout exposed to nitrosamines, which caused liver cancer, and found that I3C effectively inhibited tumor development.

**Q. You also looked at the effect of ellagic acid or chlorophyllin in preventing dimethylbenzanthracene-induced cancer in trout. What did you find?**

**A.** Dimethylbenzanthracene is a very potent synthetic carcinogen in rainbow trout that gives us the ability to study cancer in the kidney, stomach, liver, and swim bladder at the same time because it hits all those target organs. We got some curious results with I3C against that carcinogen. The dose-response relationship was particularly interesting. Ellagic acid is found in many kinds of berries—raspberries, blackberries, and black raspberries. In those experiments, we collaborated with Gary Stoner of Ohio State University. Gary had determined that ellagic acid acted as a tumor suppressing agent in esophageal carcinogenesis. That means that it's given after cancer is initiated, and you look at its effects on the further expansion and development of tumor cells. We tested ellagic acid in rainbow trout to see if it was effective as a tumor suppressing agent against stomach carcinogenesis initiated by dimethylbenzanthracene. We found that it was a pretty potent suppressing agent against stomach cancer, although the liver cancers in the same animals initiated by the same carcinogen were not affected. There are problems with ellagic acid, though. It has virtually no bioavailability—it's not taken up by the body, so it's not surprising that it wouldn't work in the liver. And that's why it's been effective in a gastrointestinal model where there's at least some access and ability to interfere with subsequent tumor development.

**Q. One of your more recent studies investigated the effect of tomatine in carcinogenesis. What is that compound and where does it come from?**

**A.** Tomatine and tomatidine are flavonoid-type compounds found in tomatoes, especially in green tomatoes. That work came about as a result of a small meeting that I go to every year called Western Regional Project W-2122. One of the members of that project is Mendel Friedman, who works at the USDA facility in Albany, California. Mendel was doing some work with tomatine and tomatidine and found them to be interesting anti-bacterial agents.

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# Dr. George Bailey Retires



**D**r. George Bailey retired at the end of February after a nearly 30-year career at Oregon State University and after 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 has published over 150 papers in peer-reviewed scientific journals and has won several awards for outstanding research, including the Sixth Prince Hitachi Prize in Comparative Oncology in 2001. Over the years, he has also mentored many graduate students at OSU.

George has worked most recently in the area of "Cancer Chemoprevention by Chlorophylls, and Basic Mechanisms of Carcinogenesis." He has been 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. Some of his early work is discussed in the interview that begins on the first page of this newsletter. Using the rainbow trout model, George and his colleagues have been 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 putative cancer chemoprotective phytochemicals. He has also assembled the most comprehensive database of carcinogen-DNA adducts as early biomarkers for cancer chemoprotection by phytochemicals in any animal model. Based on his results in trout showing that liver cancer induced by aflatoxin could be inhibited by chlorophyllin through a blocking mechanism (chlorophyllin binds to aflatoxin, preventing its uptake in the body), George and his colleagues initiated a human trial in China where people are unavoidably exposed to dietary aflatoxin. The research team measured cancer biomarkers in the urine, which were substantially decreased in people taking chlorophyllin supplements.

George was one of several members of a faculty advisory committee established by Dr. Richard Scanlan, OSU's Dean of Research in 1995, to advise OSU's then-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 will continue to think about science and fly-fish for trout in the Cascade Mountains of Oregon, but probably won't return to the surfing he enjoyed as a student in southern California. [LPI](#)

## *Continued from page 5 — Interview with Dr. George Bailey*

He suggested that it may be worthwhile to try to increase the amount of these compounds in tomatoes. And I wanted to know about the effects on cancer. Is there any risk? So we planned a trout experiment with Mendel. Tomatine turned out to be a pretty effective blocking agent. We don't know what it will do post-initiation, because we didn't have enough tomatine for that kind of experiment.

**Q. You've also done some work in trout with cooked-meat mutagens and carcinogenic polycyclic aromatic hydrocarbons produced by combustion. Did you find any dietary compounds that are chemoprotective?**

**A.** We tested chlorophyllin in trout and saw exactly the same mechanism that we saw against aflatoxin—the ability to form strong complexes between chlorophyllin and the carcinogen, resulting in a reduction in the bioavailability of the carcinogen to the whole animal. We didn't find any effect of chlorophyllin on genes important in carcinogen metabolism. In the early 1980s, I went to a conference in New Orleans, where one of the speakers was Dr. Takashi Sugimura, who discovered

the heterocyclic amines or cooked-meat mutagens. He became very interested in our work, so we arranged for my first visit to Japan to try to see if these compounds would cause cancer in rainbow trout. I was particularly interested because nobody had done a chemoprotection experiment at that time with these mutagens. Unfortunately, the four heterocyclic amines that we tested in trout by embryonic microinjection or dietary exposure were not carcinogenic. At the time, we didn't have rodent models that may have produced different results. When Rod Dashwood left my lab and went to Hawaii to begin his own career, he was able to break away from the trout model and tackle the work with rodents. And now he is back at OSU as a principal investigator in LPI.

**Q. Based on your knowledge of carcinogenesis and chemoprotection, what can we do to minimize the risk for cancer?**

**A.** When you look at the experimental animal data, it's very clear that many components of fruits and, especially, vegetables are protective in a large number of models. The isothiocyanates in cruciferous vegetables and catechins in tea that Rod has worked with are very effective in many animal models. And now some

translational research is being done in humans, especially for esophageal and oral carcinogenesis. The tea catechins look pretty effective for people at high risk. But there is a question for most of us, is there compelling evidence that a diet high in vegetables and fruits, or specific supplements, protects against most cancers? The epidemiological research at present just doesn't support that. So we have to ask, why is this so? Epidemiological studies rely on food recall questionnaires and so on, and many of us don't have a lot of faith in that because human memory is too selective—you're not gathering real data. We're really going to know with more certainty when we have a large enough body of prospective studies with either biomarkers or cancer end points. That's when we'll know the answer. Some risks can be estimated from epidemiological studies. For example, we know that

high-calorie, high-fat diets put us at increased risk for endometrial cancer and a number of other cancers.

**Q. Do you think that there's any benefit to eating organic food as opposed to food that's been grown with synthetic pesticides?**

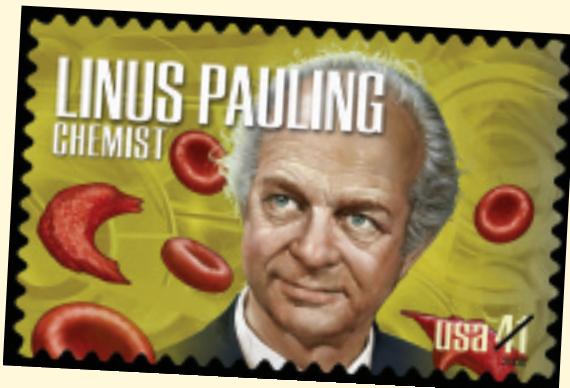
**A.** The array of putative cancer chemoprotective phytochemicals is the same in organic or non-organic food. You could avoid the foods that are not organic if you are convinced that you're at some risk from the chemicals that are used in the farming industry to produce the crop. In terms of the environment—the ecosystem—reducing the amount of synthetic pesticides would be a good strategy. To protect the environment we have to pay a cost. And the cost would be, as you know, somewhat lower levels of food production and more blemishes on the food we consume. **LPI**

## New Stamp Honors Linus Pauling

On March 6<sup>th</sup>, the United States Postal Service (USPS) issued a set of four stamps, each commemorating an American scientist. The set features theoretical physicist John Bardeen, biochemist Gerty Cori, astronomer Edwin Hubble, and Linus Pauling. The USPS press release stated, "Structural chemist Linus Pauling (1901-1994) determined the nature of the chemical bond linking atoms into molecules. He routinely crossed disciplinary boundaries throughout his career and made significant contributions in several diverse fields. His pioneering work on protein structure was critical in establishing the field of molecular biology and his studies of hemoglobin led to many findings, including the classification of sickle cell anemia as a molecular disease." The stamp honoring Pauling features an illustration of abnormal red blood cells from patients with sickle-cell anemia. In 1949 Pauling discovered that the red blood cells from such patients become sickled when their hemoglobin molecules combine with themselves in de-oxygenated blood, forming long rods that twist the cells into the characteristic shape observed in the disease. Sickle-cell anemia was the first disease to be characterized as a molecular disease.

Pauling's daughter, Linda Pauling Kamb, was present at a ceremony convened by the USPS at Madison Square Garden in New York City on March 6, the first day of issue. On the same day in Corvallis, Oregon, Pauling's son Linus Pauling Jr., his biographer Tom Hager, and Stephen Lawson of LPI spoke at a special ceremony at Oregon State University held in conjunction with the USPS. Dr. Pauling Jr. noted several experiences that his father had at Oregon Agricultural College (now OSU) that helped him be so successful. Linus Pauling Jr. also thanked

OSU for honoring his father's legacies. Both Linus Pauling Jr. and Tom Hager mentioned the government's political persecution of Pauling in the 1950s that severely hindered his scientific work and ability to win federal grant support. The denial of a passport renewal by the State Department in 1952 during the McCarthy era compromised Pauling's work on the structure of DNA, resulting in his failure to see Rosalind Franklin's X-ray crystallographic pictures of pure DNA in England. Watson and Crick, of course, had access to that critical data and discovered the double helix.



Pauling was largely vindicated by the Nobel Peace Prize in 1963. He was awarded the National Medal of Science in 1975 for "the extraordinary scope and power of his imagination, which has led to basic contributions in such diverse fields as structural chemistry and the nature of chemical bonding, molecular biology, immunology, and the nature of genetic diseases," and the new stamp is further recognition by the government of his monumental achievements. Stephen Lawson recalled Pauling as charismatic and courageous and said the 2000 "Millennium Essay" in the respected scientific journal *Nature* called Pauling one of the greatest thinkers and visionaries of the millennium, ranking alongside Galileo, Da Vinci, Newton, and Einstein. In a video clip, U.S. Representative Darlene Hooley of Oregon saluted Pauling. LPI Director Balz Frei and Cliff Mead, Head of Special Collections, which archives the Ava Helen and Linus Pauling Papers, were also acknowledged at the ceremony. Corvallis Postmaster John Herrington and postal employees were present to hand stamp souvenir first-day-of-issue envelopes that featured a unique postal cancellation in the shape of a molecular model. **LPI**



# What's Good about Dietary Fat?

Donald B. Jump, Ph.D.  
Professor of Nutrition and Exercise Sciences  
LPI Principal Investigator

We are inundated with information on diet. The balance between dietary carbohydrate, fat, and protein is a hot topic for the media. The goal of this article is to explain some basic features about dietary fat, why we need fat in our diet, and when we get into trouble with dietary fat.

## Why we need fat in our diet

Dietary fat plays several key roles in our physiology and well-being. It provides flavor to food. Ingestion of fat is important for the intestinal absorption of lipid-soluble vitamins like vitamins A, D, E, and K. Fat is a key source of metabolic energy. Components of fat are also important building blocks of all cells in the body. Fat, in the form of glycerol- and sphingolipids,

makes up the bulk of cellular membranes.

These complex lipids are composed of fatty acids bound to glycerophosphate or sphingosine. Cellular membranes serve as barriers between compartments, such as the inside and outside of the cells, and are important for the maintenance of cellular structural integrity. They also play important roles in metabolism and in the regulation of cell function. Fatty acids also function as signaling molecules, thereby regulating cell function.

## Types of fat

There are four kinds of fat: saturated, *trans*, monounsaturated, and polyunsaturated fat. These terms refer to the structure of the fatty acids found in fat. Saturated fatty acids have no double bonds between carbon atoms and are rigid molecules (see Figure). Monounsaturated fatty acids have a single double bond, while polyunsaturated fatty acids have two or more double bonds. *Trans* fats, also known as partially hydrogenated fats, are formed through the industrial processing of liquid vegetable oils. *Trans* fats are unsaturated fatty acids with the double bond in the *trans* configuration. Most naturally occurring double bonds in fatty acids are in the *cis* configuration. The double bond configuration affects the flexibility of the molecule

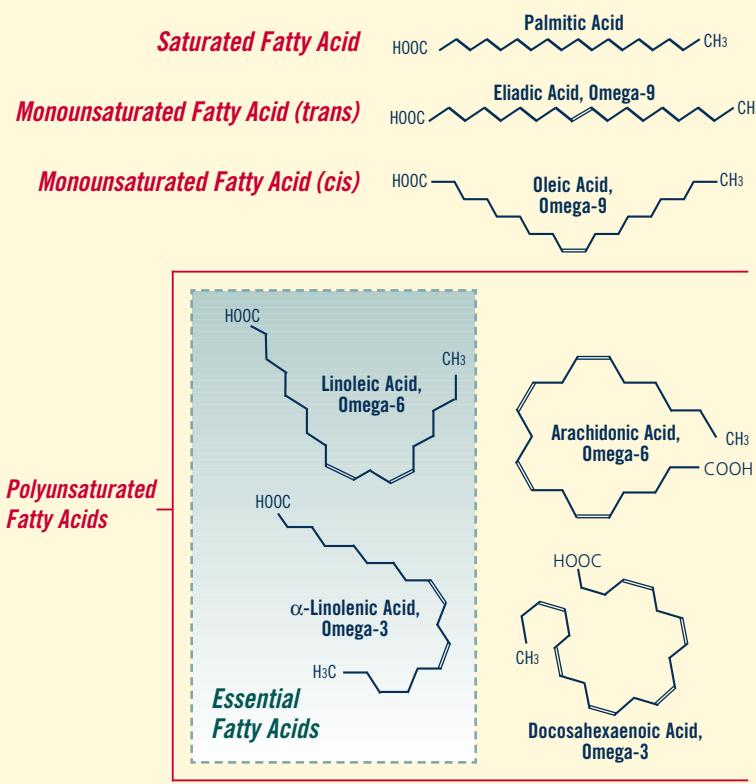
around the double bond. As such, *trans* fatty acids are rigid like saturated fats.

Unsaturated fatty acids are also grouped into classes, depending on the position of the first double bond relative to the methyl (-CH<sub>3</sub>) end of the molecule (see Figure). Omega-3, omega-6, and omega-9 are common dietary mono- and polyunsaturated fatty acids. The flexibility of these molecules increases with the number of double bonds.

Fatty acids in foods are incorporated into several types of complex lipids like triglycerides, cholesterol esters, and phospholipids. The fatty acid composition of foods is complex. For example, pork fat consists of 40% saturated fatty acids (SFA), 45% monounsaturated fatty acids (MUFA), and 15% polyunsaturated fatty acids (PUFA), while olive oil is 14% SFA, 74% MUFA, and 12% PUFA.

For us to utilize fat in food, complex lipids must first be hydrolyzed in the small intestine. The released fatty acids are absorbed by intestinal cells where they are converted to triglycerides for packaging into lipoprotein particles called chylomicrons, which circulate in the blood.

Chylomicrons are vehicles for the delivery of fat to cells. Enzymes like lipoprotein lipase on the surface of cells degrade chylomicron lipids so that fatty acids can enter cells. Once in cells, fatty acids are processed through various metabolic pathways, such as assembly into triglycerides for storage, assimilation into phospholipids for membrane synthesis, or oxidation in the mitochondria for energy production.



**Structures of common dietary fatty acids**

Although we get much of our fat requirement from the diet, we have the capacity to synthesize fat in adipose tissue and in the liver. Excess carbohydrate consumption, for example, results in the conversion of carbohydrate (glucose) to palmitic and oleic acid (see Figure); these fatty acids are assimilated into triglycerides for storage. Some fatty acids, however, cannot be synthesized in our bodies. These "essential fatty acids," linoleic acid (omega-6) and alpha-linolenic acid (omega-3), are found in vegetable oils like corn and canola. Walnuts are also a good source of alpha-linolenic acid. Essential fatty acids are precursors to the very long chain fatty acids, arachidonic acid (ARA, omega-6) and docosahexaenoic acid (DHA, omega-3) (see Figure). ARA and DHA function as both structural and regulatory molecules. Deficiency of essential fatty acids

leads to multiple pathologies associated with reproductive failure, poor visual acuity, learning disabilities, capillary fragility, skin lesions, fatty liver, and other problems. Therefore, the food industry supplements certain foods with PUFA to prevent these problems. For example, Enfamil LIPIL® from Mead Johnson Nutritionals is a popular infant formula supplemented with both ARA and DHA.

## Fatty acids are signaling molecules

In addition to serving as sources of metabolic energy and structural elements of cells, fatty acids are now recognized as signaling molecules. In many cases, fatty acids act like hormones to control cell function. ARA, for example, is a precursor for eicosanoids like prostaglandins. Prostaglandins are oxidized lipids that bind receptors on cells. Activation of prostaglandin receptors initiates signaling cascades that control many physiological functions. Eicosanoids derived from omega-6 PUFA are typically pro-inflammatory. Omega-3 PUFA like DHA and eicosapentaenoic acid (EPA), which are found in fish, interfere with the conversion of omega-6 PUFA to eicosanoids, thereby exerting anti-inflammatory functions. Moreover, when EPA and DHA are oxidized to eicosanoids and docosanooids (resolvins), they form lipids with anti-inflammatory properties.

Fatty acids also regulate gene expression, controlling the types of proteins cells make. Changes in gene expression affect metabolism, inflammation, and cell growth and division. Fatty acids bind to and activate peroxisome proliferator activated receptors, which control the expression of multiple genes affecting whole body fatty acid oxidation, storage, and inflammation. PUFA also target other proteins in cell nuclei that regulate gene expression, including nuclear factor κB (NFκB), sterol regulatory element binding protein-1 (SREBP1), carbohydrate regulatory element binding protein (ChREBP), and Max-like factor-X (MLX). NFκB controls the production of proteins involved in inflammation and immunity. ChREBP, MLX, and SREBP1 control the production of proteins regulating whole body glucose metabolism and fatty acid synthesis and storage.

Finally, cellular membranes contain microdomains called "lipid rafts." Lipid rafts harbor key proteins involved in transferring signals from outside to inside cells. Changes in the structure of lipid rafts by the composition of lipids in membranes alter cell signaling and function. Fatty acids are important regulatory molecules that use diverse routes to control multiple facets of cell metabolism, division, and differentiation, as well as inflammation.

## What is wrong with fat?

The type and quantity of fat ingested affects our health. Health problems arise when we ingest too much fat or the wrong type of fat. For example, increasing saturated and *trans* fat in the diet is associated with elevated blood LDL-cholesterol, a pro-atherogenic risk factor. The American Heart Association recommends consuming less than 7% of calories as saturated fat and less than 1% as *trans* fat. Many experts recommend eliminating *trans* fat altogether. In contrast, increasing omega-3 PUFA in the diet is anti-atherogenic, it protects against heart disease. Helpful information on the fat composition of foods can be found at <http://www.myfatstranslator.com>.

Fat is typically stored in the body's adipose tissue as triglyceride and cholesterol esters. High fat diets lead to the accumulation of fat in non-adipose tissues like liver, heart, muscle, and pancreas. The accumulation of excess fat in the heart promotes a form of cardiomyopathy, and too much fat in the liver can progress to steatohepatitis (inflamed liver), fibrosis, and cirrhosis. Accumulated fat in tissues promotes lipotoxicity, a syndrome characterized by caspase activation and programmed cell death (apoptosis) brought on by the accumulation of lipid-derived signaling molecules (diacylglycerol, ceramide, and reactive oxygen species). Too much ingested fat also interferes with the function of insulin. Insulin normally promotes glucose uptake by tissues for oxidation and storage, but impaired insulin action affects both glucose and lipid metabolism, leading to hyperglycemia and hyperlipidemia, which are risk factors associated with non-insulin dependent diabetes mellitus (NIDDM). NIDDM is the most common form of diabetes in Western societies. Complications arising from diabetes include blindness (retinopathy), heart disease, nerve damage (neuropathy), and kidney damage (nephropathy). More information on diet and diabetes can be found at the American Diabetes Association Web site, <http://www.diabetes.org>.

Just as too much dietary fat is harmful, eating the wrong type of fat is also harmful. Western diets contain too much saturated and omega-6 PUFA and too little omega-3 PUFA. The balance between omega-6 and omega-3 PUFA is important in the context of the production of pro- versus anti-inflammatory lipids. Chronic diseases like atherosclerosis and diabetic retinopathy are inflammatory diseases of the vasculature. Omega-6 PUFA are pro-inflammatory, while omega-3 PUFA—particularly EPA and DHA—are anti-inflammatory. Therefore, the balance between omega-3 and omega-6 lipids is an important determinant in the progression of chronic inflammatory diseases. Unfortunately, humans do not efficiently convert the common plant-derived omega-3 PUFA, alpha-linolenic acid, to EPA and DHA. The

American Heart Association recommends increasing omega-3 PUFA intake by consuming fish like salmon and tuna, which are good sources of EPA and DHA.

As we know, the diet is a key environmental factor affecting our health. It is also one factor that we can control. As such, a balance of fat—the amount and type—is important to promote health and prevent disease. Additional information on the role of dietary fat and health can be found on the Linus Pauling Institute Micronutrient Information Center, <http://lpi.oregonstate.edu/infocenter>. **LPI**



# Oxygen Club of California Annual Meeting

The Oxygen Club of California held its annual scientific conference, co-sponsored by LPI, in Santa Barbara, California, in mid-March. *Oxidants and Antioxidants in Biology* featured 37 presentations in six sessions: "Age-related Metabolic Pathways, Mitochondrial Nutrients, and Neurodegeneration," "Choline, Metals, Amino Acids, and Lipophilic Micronutrients in Brain Health and Function," "Flavonoids in Cell Signaling and Neuronal Function," "Oxidative Stress and Thiol Redox Circuits in Cell Function," "Lipoic Acid in Cell Signaling and Transcription," and "Clinical Studies on Lipoic Acid."

William Pardridge (UCLA) and Bert Sakmann (Max-Planck-Institute for Medical Research), winner of the 1991 Nobel Prize in Medicine or Physiology for "discoveries concerning the function of single ion channels in cells," gave the keynote lectures. Pardridge discussed the types of molecules that cross the blood-brain barrier (BBB). Only small lipid-soluble molecules diffuse across the BBB, but larger water-soluble molecules cross via three mechanisms. One of these, the receptor-mediated transport system, may be used to allow Trojan horse molecules to deliver metabolites or drugs to the brain. Sakmann described rat experiments in which energy expenditures in extremely small regions of the brain can be measured and correlated with specific actions, such as a whisker twitch, and concluded, "thoughts need food."

Several presentations focused on abnormal molecular events that characterize Alzheimer's disease. Based on its attenuation of inflammation in cell culture studies, 600 mg/day of alpha-lipoic acid were given to a small group of Alzheimer's patients for four years. While no significant effect on cognitive decline was found in moderate to severe Alzheimer's patients, those with early dementia exhibited less deterioration in cognitive function. In animal experiments, antioxidant treatment lowered oxidative stress caused by amyloid beta-peptide formation in the brain. Amyloid beta-peptide and neurofibrillary tangles are pathological abnormalities in the brain associated with Alzheimer's disease. Novel estrogen-like molecules and genistein from soy have been tested *in vitro* for their neuroprotective properties and may help prevent cognitive decline associated with estrogen deficiency. Additionally, pomegranate juice fed to mice used as a model of Alzheimer's disease reduced the formation of amyloid beta-peptide by about 50%, which was associated with improved cognitive and physical performance.

Flavonoids in blueberries and another flavonoid in strawberries called fisetin affect cell signaling pathways and improve neuronal function in the brain. However, flavonoids, such as tea catechins, are poorly absorbed into the bloodstream and extensively and rapidly metabolized. Their metabolites have not been detected in cerebrospinal

fluid, although the effects of supplementation on brain function have been repeatedly demonstrated. Flavonoids in tea and pomegranates have also been shown to inhibit the development of prostate cancer.

Alpha-lipoic acid, an effective mitochondrial antioxidant, has been used to treat diabetic neuropathy, probably by modulating cell-signaling pathways and reducing advanced glycation end products. Lipoic acid has also been found to prevent cardiomyopathy in mice. Lipoic acid fed to old rats protects them from toxicological insults by increasing the synthesis of glutathione, an important endogenous antioxidant involved in detoxification. In old rats, lipoic acid elevates the nuclear levels of the transcription factor, Nrf2, that regulates the synthesis of glutathione. When fed to old rats in combination with acetyl-L-carnitine, a non-protein amino acid, lipoic acid improves mitochondrial function, resulting in reversal of age-related declines in cognitive function and physical activity. The combination has also been found to reduce hypertension in people and to lower markers of inflammation in patients with sickle-cell anemia.

About 90 posters were also presented on topics ranging from the growth inhibition by vitamin C of human tumors implanted in mice to the relaxation of arteries by kynurenone, which is derived from the amino acid tryptophan.

Awards were given to Drs. Enrique Cadenas, Klaus Kraemer, and Steven Zeisel. LPI Principal Investigator Dr. Tory Hagen won the Outstanding Investigator Award. Additionally, Young Investigator Awards were presented to Drs. Sabine Augustin, Philip Lam, and Kit Tong. **LPI**



Photos courtesy  
of Lester Packer



# What is Vitamin D and Why Do We Need It?

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

Vitamin D is a fat-soluble vitamin that functions as a hormone in the body to regulate calcium metabolism. Together with parathyroid hormone (PTH), vitamin D tightly controls blood concentrations of calcium. For example, when serum calcium levels are low, such as when dietary calcium intake is inadequate, PTH is secreted from the parathyroid glands. PTH stimulates the activation of vitamin D from its prohormone form; active vitamin D in turn promotes intestinal absorption of calcium, mobilizes calcium from bone, and increases retention of calcium by the kidneys. These actions effectively increase serum levels of calcium. Maintaining calcium homeostasis is vital for normal functioning of the nervous system, as well as for bone growth and maintenance of bone density. Thus, vitamin D is necessary for skeletal integrity and the prevention of rickets, osteomalacia (loss of bone mineralization), and osteoporosis.

In addition to its well-known involvement in calcium balance and bone metabolism, vitamin D has a number of other physiological roles, including maintaining muscle strength, modulating immune function, and regulating blood pressure. Moreover, vitamin D functions to help regulate cellular differentiation, the biological process by which cells become specialized for a specific function. Due to these physiological functions, vitamin D has been implicated in protection against muscle pain and weakness, certain autoimmune diseases, hypertension, and even some forms of cancer.

## Vitamin D metabolism and biochemical action

Vitamin D is essentially a prohormone that is biologically inactive and therefore must be metabolized to its active form by the liver and kidneys. In the liver, provitamin D is converted to 25-hydroxyvitamin D [25(OH)D], the major circulating form of vitamin D. In the kidneys, this metabolite is further converted to the active, most potent form of vitamin D: 1,25-dihydroxyvitamin D [1,25(OH)<sub>2</sub>D]. Most of the physiological effects of vitamin D in the body are related to the hormone activity of 1,25(OH)<sub>2</sub>D. Biological effects of 1,25(OH)<sub>2</sub>D are primarily mediated through a transcription factor called the vitamin D receptor (VDR). Transcription factors are proteins that function to initiate, enhance, or inhibit the expression of certain genes. Once inside a cell's nucleus, 1,25(OH)<sub>2</sub>D binds to the VDR and, along with other cofactors, initiates a cell-signaling cascade that ultimately modulates gene expression. More than 50 genes in tissues throughout the body are regulated by 1,25(OH)<sub>2</sub>D.

## What are sources of vitamin D?

Vitamin D is a unique nutrient because its requirements can be met not only by dietary intake, but also from exposure to sunlight. Humans can synthesize vitamin D<sub>3</sub> (cholecalciferol) in skin cells following exposure to ultraviolet-B radiation. In fact, sunlight exposure can provide most people with their entire vitamin D requirement, although people living in Northern latitudes cannot produce sufficient vitamin D during the winter months. Additionally, individuals with dark-colored skin synthesize markedly less vitamin D on exposure to sunlight than those with light-colored skin. The elderly also have diminished capacity to synthesize vitamin D from sunlight exposure. Further, using sunscreen blocks endogenous production of vitamin D.

When sun exposure is insufficient for the synthesis of adequate amounts of vitamin D in the skin, obtaining vitamin D from foods or supplements is essential for health. However, very few foods naturally contain vitamin D. The richest sources of vitamin D include oily fish, such as mackerel, salmon, or sardines; fish liver oils; and eggs from hens that have been fed vitamin D. In the U.S., milk and infant formula are fortified with vitamin D so that they contain 400 IU (10 mcg) per quart. However, other dairy products, such as cheese and yogurt, are not always fortified with vitamin D. Certain cereals, breads, soy and rice milks, as well as orange juice, may be fortified with vitamin D. Today, products sold in the U.S. are typically fortified with vitamin D<sub>3</sub> instead of vitamin D<sub>2</sub> (ergocalciferol), a form synthesized by fungi and plants. A few studies indicate that vitamin D<sub>2</sub> may be less active in humans than vitamin D<sub>3</sub>.

## Measurement of vitamin D status

Increased exposure to sunlight or increased dietary intake of vitamin D increases serum levels of 25(OH)D, making the serum 25(OH)D concentration a useful clinical indicator of vitamin D status. There is general agreement that serum 25(OH)D level is the best indicator of vitamin D status; however, the cutoff values have not been clearly defined. Some experts believe that current laboratory reference ranges, with 20-25 nmol/L (8-10 ng/mL) as the cutoff for severe deficiency, may be too low. Research suggests that PTH levels and calcium absorption may not be optimized until serum 25(OH)D levels reach approximately 80 nmol/L (32 ng/mL). It has been estimated that vitamin D intakes of at least 800 to 1,000 IU/day are required by adults living in temperate latitudes to achieve serum 25(OH)D levels of at least 80 nmol/L.

## Vitamin D deficiency

Inadequate vitamin D from sun exposure or dietary intake leads to deficiency states. Severe vitamin D deficiency has serious consequences for bone health, manifesting as rickets in children and osteomalacia in adults. Vitamin D deficiency during infancy or childhood results in failure of bone to mineralize, thereby leading to weak, softened bones and the skeletal disorder called rickets. Malformed bones, such as bowed legs, are a hallmark of rickets. In infants, rickets may result in delayed closure of the fontanelles (soft spots) in the

*continued on page 12*

## *Continued from page 11 — What is Vitamin D*

skull, rib cage deformities, and seizures induced by low levels of serum calcium. Although fortification of foods has led to complacency regarding vitamin D deficiency, nutritional rickets is still being reported throughout the world. Severe vitamin D deficiency in adults leads to a condition known as osteomalacia, which is characterized by weak muscles and bones as well as bone pain. These symptoms result from progressive loss of bone mineralization.

Today, less obvious states of vitamin D deficiency are actually quite common in both children and adults. Such milder vitamin D deficiencies increase the risk of osteoporosis and possibly other health problems, including multiple sclerosis, rheumatoid arthritis, hypertension, cardiovascular disease, and certain types of cancer.

## Risk factors for vitamin D deficiency

There are several factors that place individuals at risk for vitamin D deficiency. Infants who are exclusively breast-fed are at high risk of vitamin D deficiency because human milk generally does not provide sufficient vitamin D. Older infants and toddlers exclusively fed milk substitutes and weaning foods that are not vitamin D fortified are also at risk of vitamin D deficiency. On the advice of the medical community, parents often use sunscreen on their children. Sunscreen effectively blocks skin synthesis of vitamin D. For instance, applying sunscreen with a sun protection factor (SPF) of 8 reduces vitamin D production by about 95%. Likewise, individuals who cover all exposed skin whenever outside (e.g., for religious or cultural reasons) are at risk for vitamin D deficiency. People with dark-colored skin have a reduced ability to synthesize vitamin D upon exposure to sunlight than those with light-colored skin. Elderly individuals also have a lower capacity to synthesize vitamin D on exposure to ultraviolet-B radiation and often stay indoors. Institutionalized adults who are not supplemented with vitamin D are at extremely high risk of vitamin D deficiency. Additionally, individuals with inflammatory bowel disease or fat malabsorption syndromes are at a heightened risk for vitamin D deficiency. Further, obesity increases the risk of vitamin D deficiency because obese individuals cannot easily access the vitamin D stored in body fat.

## Vitamin D toxicity

Vitamin D toxicity, called hypervitaminosis D, causes abnormally high serum levels of calcium (hypercalcemia). If untreated for long periods of time, this can result in bone loss, kidney stones, and calcification of organs. Hypercalcemia has been observed following daily doses of greater than 50,000 IU of vitamin D. However, the Food and Nutrition Board of the Institute of Medicine conservatively established the tolerable upper intake level (UL) for vitamin D at 2,000 IU/day (50 micrograms/day). Many experts believe that vitamin D toxicity is very unlikely in healthy people at intake levels below 10,000 IU/day.

Yet, certain medical conditions can increase the risk of hypercalcemia in response to an increase in vitamin D

nutrition. Such conditions include primary hyperparathyroidism, sarcoidosis, tuberculosis, and lymphoma. Patients with these medical conditions should consult a qualified health care provider regarding any increase in vitamin D intake.

## Sun exposure controversy

Sun exposure can provide most people with their entire vitamin D requirement, but the same radiation that stimulates vitamin D synthesis in skin can also cause skin damage. Ultraviolet light has been linked to melanoma and non-melanoma forms of skin cancer, but the causes of skin cancer appear to be multifactorial. Due to concerns of skin damage and cancer, many people use sunscreen or protective clothing whenever outdoors; others avoid the sun altogether.

However, several recent studies have reported that vitamin D may protect against various internal cancers. Results of some, but not all, studies suggest that higher 25(OH)D blood levels may be beneficial against development of colorectal, breast, and prostate cancers. Vitamin D is known to be important in preventing osteoporosis and may also have a role in preventing hypertension and autoimmune diseases.

Given the fact that vitamin D deficiency is so widespread and that vitamin D may protect against certain diseases, moderate exposure to sunlight may indeed be sensible. Application of sunscreen after 10 to 15 minutes in the sun would block any deleterious effects of ultraviolet light.

## Dietary intake recommendations

In 1997, the Food and Nutrition Board of the Institute of Medicine set adequate intake (AI) levels that assume no skin synthesis of vitamin D. The AI values (see Table below) reflect vitamin D intakes likely to maintain serum 25(OH)D levels of at least 37.5 nmol/L (15 ng/mL). While the AI level may help individuals avoid overt deficiency, many experts feel these dietary intake recommendations are inadequate. The LPI recommends that healthy adults take a multivitamin supplement daily that supplies 400 IU of vitamin D. For people over the age of 50, an additional 400 IU/day is recommended. [LPI](#)

Adequate Intake (AI) for Vitamin D

Life Stage	Age	Males mcg/day (IU/day)	Females mcg/day (IU/day)
Infants	0-12 months	5 mcg (200 IU)	5 mcg (200 IU)
Children	1-13 years	5 mcg (200 IU)	5 mcg (200 IU)
Adolescents	14-18 years	5 mcg (200 IU)	5 mcg (200 IU)
Adults	19-50 years	5 mcg (200 IU)	5 mcg (200 IU)
Adults	51-70 years	10 mcg (400 IU)	10 mcg (400 IU)
Adults	>70 years	15 mcg (600 IU)	15 mcg (600 IU)
Pregnancy	all ages	—	5 mcg (200 IU)
Breast-feeding	all ages	—	5 mcg (200 IU)

Abbreviations: mcg = microgram, IU = international unit

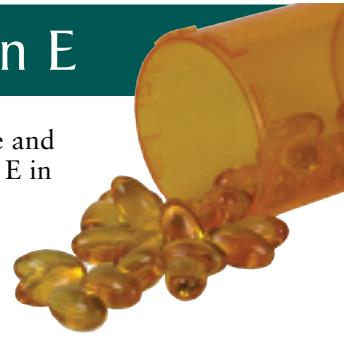
# "Fatally Flawed" Clinical Trials of Vitamin E

Over 60 years ago, E. V. Shute of Canada wrote a letter about the effectiveness of vitamin E in treating coronary heart disease that was published in the scientific journal *Nature*. Anecdotal evidence and clinical reports continued to emerge for decades, and Linus Pauling periodically wrote about the health benefits of vitamin E supplements. It wasn't until 1993 that the conventional medical community became excited, when two reports were published in *The New England Journal of Medicine* by Harvard researchers, who reported that their epidemiological studies found associations between supplemental vitamin E intake and decreased risk for coronary heart disease in men and women. Those observational studies stimulated randomized clinical trials of vitamin E and heart disease, some of which have not substantiated a reduced risk for clinical events, such as strokes, heart attack, or death, in patients with heart disease. All of the clinical trials published thus far have looked at vitamin E supplements in treating heart disease, and none has examined the long-term use of vitamin E supplements in healthy people to prevent heart disease. The only evidence we have on that score comes from observational studies like the two mentioned above by the Harvard group. The use of vitamin E to prevent or treat heart disease remains controversial.

In the Spring/Summer 2002 LPI Research Newsletter, Jeffrey Blumberg of Tufts University contributed an invited article, "Unraveling the Conflicting Studies on Vitamin E and Heart Disease," in which he suggested that results of vitamin E studies have been problematically influenced by a variety of issues, including inadequate dose, duration, suboptimum form, and polypharmacy (the use of multiple drugs) of patients. He also noted that none of the clinical studies had measured oxidative stress in the subjects—an important shortcoming since the putative benefit of vitamin E is ascribed to its role as an antioxidant. If no measurements of oxidative stress were recorded, it was impossible to determine if supplemental vitamin E had the desired effect of attenuating oxidative stress. Most studies have also failed to measure plasma vitamin E to determine if it was increased after supplementation.

In a recent commentary published in *Free Radical Biology & Medicine*, Blumberg and LPI's Balz Frei discussed the results of a research paper by L. Jackson Roberts *et al.* in that same issue of the journal. Roberts *et al.* found that very high daily doses of vitamin E (1,600 IU or 3,200 IU d-alpha-tocopherol) taken for at least 16 weeks were required to ameliorate oxidative stress, which was assessed by the formation of F<sub>2</sub>-isoprostanes, in hypercholesterolemic men and women. F<sub>2</sub>-isoprostanes are formed in the body by the oxidation of arachidonic acid, an omega-6 poly-unsaturated fatty acid that is essential in human nutrition. F<sub>2</sub>-isoprostanes, then, are considered biomarkers of oxidative stress. Roberts *et al.* found a decrease in plasma F<sub>2</sub>-isoprostanes at vitamin E doses of 400 IU/day and above, but statistically significant decreases were evident only at the highest doses, 1,600 and 3,200 IU/day. These doses decreased plasma F<sub>2</sub>-isoprostanes by about 35% and 50%, respectively. Blumberg and Frei noted that most trials of vitamin E and heart disease may be "fatally flawed"

because of insufficient dosage and that "the paradox of vitamin E in CVD might be resolved by studies initiated at earlier stages, of longer durations, and using higher doses and more bioavailable forms of alpha-tocopherol [vitamin E]."<sup>1</sup> Interestingly, Linus Pauling advocated taking daily doses of up to 1,600 IU of vitamin E in his book, *How to Live Longer and Feel Better*, published over 20 years ago. Even earlier, in an article for *Executive Health* in 1973, Pauling discussed the potential value of high-dose vitamin E taken for at least several months. Currently, the tolerable upper intake level for vitamin E is 1,500 IU/day of RRR-alpha-tocopherol (d-alpha-tocopherol or natural vitamin E), based on the possible risk for hemorrhage at higher doses because of impaired blood clotting.



In future clinical trials, Blumberg and Frei call for assessing the level of oxidative stress in subjects, as measured by appropriate biomarkers, in order to determine the effect of supplemental vitamin E and other antioxidants on oxidative stress. They also emphasize that investigators must pay careful attention to dose, duration, and possible drug-nutrient interactions. When such properly designed clinical trials have been conducted, we will have a much better understanding of the role of antioxidants, especially vitamin E, in heart disease. **LPI**

## LPI Pilot Project Grants

The LPI Pilot Project Grants are designed to stimulate innovative research by Oregon State University scientists relevant to the mission of the Institute. These one-year awards, made possible by financial support from LPI donors, enable investigators to obtain preliminary results that can be used to support research grant applications to federal funding agencies for more extensive projects. Last year, the awards were increased from \$20,000 to \$25,000.

### Three proposals were funded in 2008:

- Jane Ishmael (Department of Pharmaceutical Sciences) and Kathy Magnusson (Department of Biomedical Sciences) for "Dietary zinc levels influence NMDA receptor properties in the elderly"
- Carmen Wong (Department of Nutrition and Exercise Sciences) for "Protective effects of green tea in suppressing autoimmune type 1 diabetes"
- Robert Tanguay (Department of Environmental and Molecular Toxicology) for "Epigenetic modulation of micro RNAs expression during embryonic development"

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

# **40<sup>th</sup> Anniversary of “Orthomolecular Psychiatry”**

This year marks the 40<sup>th</sup> anniversary of Linus Pauling's seminal paper "Orthomolecular Psychiatry," in which Pauling introduced the term orthomolecular medicine and provided its scientific basis. Published in the highly regarded scientific journal *Science* in April 1968, the paper argued for the application of vitamins to treat mental illness. Pauling had worked on problems in mental illness since the mid-1950s, believing that abnormalities in enzyme function may help explain schizophrenia and other mental illnesses because the brain is exquisitely sensitive to its constituent chemicals. For example, chronic deficiencies of niacin or vitamin B<sub>12</sub>, resulting in pellagra or pernicious (or megaloblastic) anemia, respectively, are accompanied by mental impairment like psychosis and dementia, as well as physical symptoms. When vitamin B<sub>12</sub> deficiency was more common in the early 20<sup>th</sup> century, affected individuals often exhibited "megaloblastic madness." Indeed, Pauling's mother Belle suffered from this and succumbed in 1926 to the disease. Chronic deficiency of another B vitamin, biotin, can also cause neurological symptoms like depression and hallucinations. Even a chronic deficiency of vitamin C can result in depression.

Pauling suggested that supplying certain vitamins in amounts much larger than those required to prevent the associated deficiency diseases may help normalize brain biochemistry, resulting in attenuation of mental illness. In his paper Pauling discussed how large amounts of vitamins may enhance biological activity and cited work by Beadle and Tatum showing that the growth rate of vitamin-requiring mutant strains of *Neurospora*, a genus of fungi, actually surpassed that of the normal, vitamin-synthesizing parental strain when the vitamin was supplied in the culture medium in large amounts. Pauling also noted that the rates of enzyme-catalyzed reactions, which may be impaired in mental illness because of defective enzymes, may be normalized by supplying large amounts of the substrate, such as vitamins. For example, in a 2002 paper in the *American Journal of Clinical Nutrition*, Dr. Bruce Ames, recipient of the 2001 LPI Prize for Health Research, discussed the vitamin remediation of about 50 genetic diseases caused by poor affinity of a mutant enzyme for its coenzyme. Additionally, genetic polymorphisms (slight differences in genes among people) affect enzyme-coenzyme reactivity.

In his book *How to Live Longer and Feel Better* (20<sup>th</sup> anniversary edition from the Oregon State University Press, 2006), Pauling provided a succinct definition of orthomolecular medicine: "the preservation of good health and the treatment of disease by varying the concentrations in the human body of substances that are normally present in the body and are required for

health." An abbreviated definition might be "the right molecules in the right amounts," since *ortho* is a prefix meaning right or correct. Clearly, Pauling had vitamins and nutritionally essential minerals in mind, but his definition also encompasses phytochemicals (chemicals from plants that may affect health) and other constituents of the diet. These concepts form the basis of the research mission of the Linus Pauling Institute, which is to:

- Determine the function and role of vitamins and essential minerals (micronutrients) and chemicals from plants (phytochemicals) in promoting optimum health and preventing and treating disease,
  - Determine the role of oxidative and nitrate stress and antioxidants in human health and disease,

and, continuing the humanitarian interest and spirit of Linus Pauling, to:

  - Help people everywhere achieve a healthy and productive life, full of vitality, with minimal suffering, and free of cancer and other debilitating diseases.

For more information on orthomolecular medicine, please see "What is Orthomolecular Medicine" by Stephen Lawson in the Fall/Winter 1999 LPI Research Report, archived online at <http://lpi.oregonstate.edu/fw-99/orthomolecular.html>. **LPI**

## **Orthomolecular Psychiatry**

Varying the concentrations of substances normally present in the human body may control mental disease.

Lenn. Pauling

The methods principally used now for treating patients with mental disease are psychotherapy, psychotropics, and related efforts to provide insight and to deaease environmental stress, chemotherapy (mainly with the use of psychotropic drugs, such as chlorpromazine, or powerful sedatives from plants, such as camomile), and survival therapy. Insulin coma therapy, psychosomatic shock therapy, have reached the conclusion, through experiments summarized in the following paragraphs, that another general method of treatment, which may be called *molecular therapy*, may be found to be of great value, and may even set to be the best method of treatment for many patients.

Orthomolecular psychiatry characterizes the treatment of mental illness by the provision of the optimum molecular environment for the mind, especially the optimum concentrations of substances normally present in the human body (3). An example is the treatment of phenylketonuria children by a diet containing normal amounts of the amino acid phenylalanine. Phenylketonuria (PKU) results from a genetic defect that leads to a decreased amount or efficiency of the enzyme catalyzing the oxidation of phenylalanine to tyrosine. The patient on a normal diet

controlled synthetic mechanisms, and for essential nonsterile hormones, essential amino acids, essential fatty acids, different from the minimum daily amounts required for life or the "recommended" average daily amounts suggested for good health. Some of following paragraphs.

Evolution and Natural Selection

The process of evolution does not necessarily result in the normal progression of apparent molecular communications. Let us use ascorbic acid as an example. Of the mammals that have been studied in this respect, the only species that had the power to synthesize ascorbic acid and that also ingested it in the diet are man, some other Primates (baboon, monkey, Formosan langur monkey), and ring-tail lemur (capuchin monkey), the primate (*Papio ursinus*) (7). Presumably because of the great or greater controlling systems of the enzymes or enzymes involved in the conversion of glucose to ascorbic acid occurred some 20 million years ago in the common ancestor of man and other Primates, and moreover independently for the gorilla pig, and such cases as the bat and owl monkey.

10

## **System Molecular Conventions**

Several arguments may be advanced in support of the thesis that the optimi-



# Developments

Scott Palmer  
LPI Director of Development

## A Truly Lasting Legacy

Ralph is nothing if not resilient. He has outlived two wives, and for more than twenty years he has struggled with respiratory problems that likely would have left other people his age confined to a chair with an oxygen tank. On top of all that, he has had more than his fair share of other health issues.

A couple of months ago over lunch, he talked about his beloved Southern California and his experiences when he was a much younger man of 70. He started taking vitamin C years ago and can recall with great clarity the first time he heard Dr. Linus Pauling talk at Caltech in Pasadena. Today Ralph lives in Birmingham, Alabama, just a few doors down from his younger brother, who is only 86. When he moved to Birmingham a couple of years ago he decided to buy a house, rather than rent an apartment, because owning a home is a better long-term investment than renting. Ralph is 97.

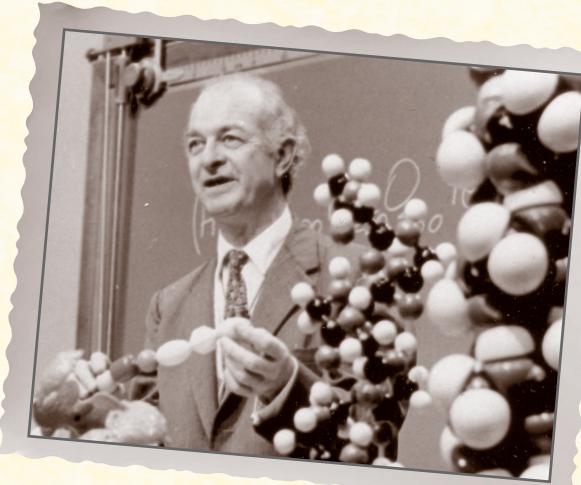
We are grateful to Ralph for many reasons. Several years ago, he provided for the Institute in his estate plan by establishing a Charitable Remainder Trust and naming LPI as one of the two beneficiaries of his trust. Last year he arranged for a Charitable Gift Annuity through the OSU Foundation, and last January, Ralph also agreed to become the first official member of the Linus Pauling Legacy Circle.

Many of LPI's friends and donors, like Ralph, have included the Institute in their estate plans. Since coming to Oregon State University in 1996, the Institute has received more than \$11,000,000 in charitable gifts from wills and trusts. For the Institute, these remarkable gifts have been transformational, providing us with critical resources to grow and enabling our scientists, like Tory Hagen, to explore new areas of research, leading to the establishment of our new Healthy Aging Program.

We understand and greatly appreciate the significance of including the Linus Pauling Institute as a beneficiary in a will or trust, and we have established the Linus Pauling Legacy Circle as a way to honor and thank this unique group of our friends for their extraordinary support.

The Linus Pauling Institute will maintain a list of Linus Pauling Legacy Circle members in perpetuity. The membership of the Legacy Circle will be published periodically in the LPI Research Newsletter, and the Institute will maintain a special plaque in the new Linus Pauling Science Center to recognize and list all members when the building is completed in 2010.

Becoming a member of the Linus Pauling Legacy Circle is simple. Our friends and donors who provide for the Institute through their will, living trust, charitable trust, gift annuity, retirement plan, or as a beneficiary of their life insurance plan automatically qualify for membership in the Linus Pauling Legacy Circle by notifying us of their decision and completing a simple enrollment form. Linus Pauling Legacy Circle members will receive a certificate acknowledging their support and a special hard-bound, commemorative twentieth anniversary limited edition of Pauling's best-selling book, *How to Live Longer and Feel Better*.



Additionally, Legacy Circle members will be invited to attend LPI events and conferences as guests of the Institute and will enjoy a standing invitation to visit LPI to see our labs and the Ava Helen and Linus Pauling Special Collection in OSU's Valley Library. For a limited time, Legacy Circle members who meet certain conditions and criteria also have the opportunity to name a room in the new Linus Pauling Science Center.

So it is with great pleasure that we announce the Linus Pauling Legacy Circle and welcome our first member—Mr. Ralph Chilton. We greatly appreciate his support.

If you would like more information about the Linus Pauling Legacy Circle or how to include the Linus Pauling Institute in your will or estate plan, please contact me at (503) 553-3407 or by email at [Scott.Palmer@oregonstate.edu](mailto:Scott.Palmer@oregonstate.edu). **LPI**

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

Mark Eisner

Joseph and Etel Thomas

Ruth Kilby

Alfred Schroeder

Dewey Edelman



# GIVING to the Linus Pauling Institute

Gifts in support of research efforts can be made at any time. Checks should be payable to *OSU Foundation for Linus Pauling Institute*. Information on giving is available through the OSU Foundation, 1-800-354-7281, or by writing to the Institute.

## Micronutrient Research for Optimum Health

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## LPI Public Lecture

Dr. Lenore Arab, professor in the David Geffen School of Medicine at UCLA, presented the 2008 LPI Public Lecture, "Personalizing Your Nutrition," on April 9th. The LPI Public Lecture series is designed to allow internationally renowned scientific and medical authorities to present reviews of important health subjects for the lay public.

Dr. Arab discussed dietary strategies to achieve our genetic potential and reduce the risk of disease, especially cancer. There is quite a lot of genetic variation among people, and the environment, including diet, can influence how genes are expressed. For example, the "copy cat" clone of a kitten that shares identical genes developed into a cat with a very different phenotype—its physical and psychological characteristics were different from those of its "twin," illustrating that dietary and other environmental factors influenced its gene expression and development. Dr. Arab also noted that consuming a high carbohydrate diet or a high protein diet dramatically changes gene expression in white blood cells in humans.

Dr. Arab recommended a diet based on plant foods, getting 30-60 minutes of exercise each day to maintain lean body mass, and limiting intake of salt, alcohol, and red meat. She stressed the need to record family history of disease and the importance of choosing diets that may help prevent disease versus diets for those who may already have disease. She also recommended avoiding the direct-to-consumer genetic testing kits because of their inaccuracies and potential for harmful disclosure that may affect insurance, employment, and family members.

## Look for these informative articles inside!

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

Page 1 ..... *Cancer Chemoprotection —*

*An Interview with George Bailey, Ph.D.*

Page 2 ..... *Inauguration of the Burgess and Elizabeth Jamieson Chair in Healthspan Research*

Page 6 ..... *Dr. George Bailey Retires*

Page 7 ..... *New Stamp Honors Linus Pauling*

Page 8 ..... *What's Good about Dietary Fat?*

Page 10 ... *Oxygen Club of California Annual Meeting*

Page 11 ... *What is Vitamin D and Why Do We Need It?*

Page 13 ... *"Fatally Flawed" Clinical Trials of Vitamin E*

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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.*



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