



OREGON STATE UNIVERSITY

The Linus Pauling Institute

RESEARCH NEWSLETTER



From the Director

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

This year we celebrate the 10th anniversary of the Linus Pauling Institute at Oregon State University. LPI was founded in California in 1973 by Linus Pauling and two associates and moved to OSU in the summer of 1996. Ever since, LPI's mission has been 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; to determine the role of oxidative and nitrative stress and antioxidants in human health and disease; and to help people everywhere achieve a healthy and productive life, full of vitality, with minimal suffering, and free of cancer and other debilitating diseases.

We have been very fortunate to attract superb scientists to LPI to carry out research relevant to our mission and now have ten principal investigators who are funded by research grants from the National Institutes of Health and other sources. While the government grants are important to our success, support from our donors continues to be vital. Your support has allowed us to establish a number of crucial programs at LPI, including LPI Pilot Projects, which award grants to researchers to obtain initial results that can be used in applying for larger, extramural grants; the Micronutrient Information Center, which is an online resource for accurate, up-to-date, and peer-reviewed information on vitamins, minerals, phytochemicals, and other constituents of the diet (<http://lpi.oregonstate.edu/infocenter/>); the *LPI Research Newsletter*; scientific seminars and public lectures; and our biennial Diet and Optimum Health Conference.

We celebrated the 10th anniversary at the beginning of November with several events here in Corvallis, including an open house and a full day of scientific seminars by our principal investigators highlighting our research. The speakers and their presentations were as follows:

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Fighting Cancer with Phytochemicals

*An interview with David E. Williams, Ph.D.
Professor of Environmental and
Molecular Toxicology
LPI Principal Investigator*

Q. *How did you get interested in cancer prevention by dietary phytochemicals?*

A. I've always been very interested in the effect of environmental chemicals on health, going back to my undergraduate days at Reed College in Portland. We had to do an undergraduate thesis, and it was not long after Rachel Carson's book "Silent Spring" had come out on the effect of insecticides on wildlife. That got me really interested in environmental chemicals and inspired my thesis. As my career developed I came to realize a couple of things: many of our environmental exposures are through food (what we eat and drink) and that one of the most exciting approaches is a potential for prevention of diseases related to food-borne toxic chemicals. That's what got me started in the whole area.

Q. *How do you study cancer prevention by dietary phytochemicals?*

A. You have to use a model. It's very difficult to design and undertake large prospective studies with humans where you provide a dietary phytochemical and then follow them for many years. That's very expensive, involves a large number of people, and sometimes you get equivocal results. So a good animal model can provide initial information on molecular mechanisms and efficacy. We've used rainbow trout as a model for a number of years, and it's turned out to be very rewarding. That was initiated by George Bailey and others, but I've adopted that model and used it to look at the effects of phytochemicals in cancer prevention. We've also used other animal models when appropriate.

Q. *Why do you use trout?*

A. In the early 1960s there was a large outbreak of liver cancer in rainbow trout in hatcheries on the West Coast. The researchers discovered that aflatoxin was present in

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— Designated by the National Institutes of Health as a —

Center of Excellence for Research on Complementary and Alternative Medicine

In Memoriam



JANE HIGDON

(1958-2006)

The Linus Pauling Institute mourns the loss of Jane Higdon, Ph.D., who died in a bicycle accident near Eugene, Oregon, on May 31, 2006. Jane began working at LPI as a research associate in 2000. She developed and managed the LPI Micronutrient Information Center (MIC), which is a premier online resource for up-to-date, scientifically accurate, and peer-reviewed information on micronutrients, phytochemicals, and other constituents of the diet. The MIC features 54 articles written by Jane over the last six years, including sections on the 13 vitamins, nutritionally relevant minerals, vegetables, fruit, coffee, tea, lipoic acid, carnitine, coenzyme Q10, and phytochemicals like carotenoids, flavonoids, and chlorophyll. The sections on vitamins and minerals were published in 2003 in Jane's book, *An Evidence-based Approach to Vitamins and Minerals: Health Benefits and Intake Recommendations*. Jane's second volume, *An Evidence-based Approach to Dietary Phytochemicals*, will be published by Thieme Medical Publishers in late 2006.

Jane earned an A.B. in human biology from Stanford University, a nursing degree from Pace University, and two degrees from Oregon State University: a master's in exercise physiology and a doctorate in nutrition. With this diverse background and exceptionally broad knowledge, she was perfectly equipped to create the MIC. She contributed many articles to the *LPI Research Newsletter* on topics such as cruciferous vegetables, osteoporosis, tea, and how to choose a multivitamin/mineral supplement. She also co-authored a number of original scientific papers on fish oil supplementation in postmenopausal women and several comprehensive review articles, including two published

in *Critical Reviews in Food Science and Nutrition*: "Tea catechins and polyphenols: health effects, metabolism, and antioxidant functions" (2003) and "Coffee and health: a review of recent human research" (2006). The tea article has been widely cited in the scientific literature. Jane was prized at LPI for her compassionate responses to thousands of questions from the public about the role of micronutrients in health and disease.

An accomplished scholar, Jane was also highly regarded for her competitive athleticism. She spent many hours swimming, bicycling, and competing in marathons and triathlons and especially enjoyed summer bicycling vacations in Europe with her husband. Jane was truly a paragon of health who lived by her own advice: eat a healthful diet and get plenty of exercise.

Memorial services were held in Eugene and Corvallis, Oregon, in June. Many of her sporting companions spoke about her perfectionism and desire to be the best that she could be, and her colleagues in the Linus Pauling Institute and Oregon State University praised her for her exceptional scholastic talents. Dr. Balz Frei of LPI recalled her as "unusually intelligent, committed, compassionate, and truly remarkable." Dr. Tony Wilcox, Chair of the Department of Nutrition and Exercise Sciences, cited her unassuming, thoughtful, disciplined, and exuberant nature. Steve Lawson of LPI praised her as earnest, extremely competent, reliable, knowledgeable, and skeptical, "an excellent quality in a scientist." Jane was a cherished member of LPI whose legacy is embodied in the outstanding achievement of the popular and highly regarded Micronutrient Information Center. LPI has created The Jane V. Higdon Memorial Fund to endow the MIC and continue the tradition of excellence established by Jane Higdon. She is survived by her husband, Tom Jefferson; her parents; two brothers; and four sisters.

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Continued from cover — From the Director

Joseph Beckman: "Free radicals, antioxidants, and Lou Gehrig: The brain is a terrible thing to waste"

Tory Hagen: "Increasing 'healthspan' through age-essential micronutrients"

Fred Stevens: "Bioconjugation of lipid peroxidation products: A new role for vitamin C?"

George Bailey: "Chlorophylls in the prevention of cancer: From animal models to humans"

Rod Dashwood: "Genetic and epigenetic approaches to cancer prevention and therapy by dietary agents"

David Williams: "Dietary supplementation of mothers during pregnancy and nursing protects their offspring from cancer in later life"

Emily Ho: "Dietary influences on DNA integrity and prostate cancer prevention"

Tammy Bray: "Antioxidants, inflammation, and diabetes"

Maret Traber: "Everything and much more than you ever wanted to know about vitamin E"

Balz Frei: "How to live longer and feel better—what we have learned in the last twenty years"

At the banquet on November 1st, Dr. Linus Pauling, Jr. and I welcomed five new members into the Linus Pauling Institute Society, which was established in 2005 to recognize individuals who have made outstanding contributions to the welfare and success of LPI. Jeanne Rousseau, George Whatley, and Burgess and Libby Jamieson were recognized as new members, and Jane Higdon was inducted posthumously.

Finally, I am very pleased to announce that we have recruited an excellent scientist, Dr. Victoria Drake, to manage our Micronutrient Information Center, beginning in September. Victoria earned her B.A. in biology from Grinnell College in Iowa and completed her Ph.D. in nutritional sciences at the University of Wisconsin-Madison. She has published a number of papers in toxicology and on the impact of aging on the response to environmental and oxidative stress. Victoria will write new sections for the MIC, update sections written by the late Jane Higdon, and contribute articles to this Newsletter.

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the diet they were feeding to the fish. Aflatoxin was known to be a potent human carcinogen produced by a mold that grows on corn and peanuts, especially in hot, humid environments. It's not much of a problem in the U.S., except maybe in the Southeast, but it's a significant health problem in other parts of the world like Africa and, especially, China. Aflatoxin targets the liver. The trout is a very good model because trout metabolize aflatoxin the same way humans do, resulting in the same DNA adduct, the same oncogene mutation, and the same type of tumor—hepatocellular carcinoma. So there are a lot of similarities between trout and humans. In fact, in this case, trout is a better animal model than any rodent.

Q. What's a DNA adduct?

A. A DNA adduct is a covalent bond between the carcinogen and one of the chemical bases in the double strand of DNA. There are a number of adducts formed with a carcinogen like aflatoxin, and some are particularly mutagenic and carcinogenic. Aflatoxin forms a covalent bond with guanine, one of the DNA bases, and that seems to be the adduct that's especially toxic or produces mutations very readily. Those mutations can cause the cell to commit suicide or can lead to cancer.

Q. Are there economic or statistical advantages to using trout?

A. Certainly. That's something that we exploit all the time. Typically, the per diem cost for a rat or mouse is about 25 to 50 cents, but it's about a penny a day for the rainbow trout. With trout, we are able to do very large cancer studies with high statistical power and at relatively low cost. For example, we're doing a cancer study with thousands of trout that would have cost about \$7 million if we had used a mouse model and about \$14 million if we'd used a rat model. Again, that allows us to address statistically very challenging questions that you just can't accomplish with a rodent model.

Q. What carcinogens have you tested in trout?

A. That's a very good question because there are some limitations and some strengths in this model. One of the limitations is that we can't study some human cancers like breast cancer, lung cancer, or prostate cancer in trout. But trout are good for studying aflatoxin, most of the other mycotoxins, and another important class of environmental carcinogens called polycyclic aromatic hydrocarbons, or PAHs, which are formed from the combustion of any organic material. When oil-derived products, coal, or anything organic are burned, these PAHs are formed. There is a significant amount of PAHs in our diet—they're just everywhere. There are a few chemical classes of carcinogens to which trout don't respond, such as heterocyclic amines, or cooked-meat mutagens, found in proteinacious food cooked at high temperatures.

Q. How do you design experiments to study cancer prevention in trout?

A. We usually do a co-exposure in which the carcinogen and presumably protective phytochemical are given together in the diet. That's possible because we use a purified diet that we make up ourselves called the Oregon Test Diet.

Trout hatcheries around the country now use that diet. It's a purified diet with casein as the protein and a defined vitamin and mineral mix and fish oils. We can just mix the carcinogen and the phytochemical together in the diet and feed it to the trout, or we can give the phytochemical for a week or two before we expose them to the carcinogen.

Q. How do you decide which phytochemicals to test?

A. Sometimes it's just a guess. Sometimes we identify likely candidates from the scientific literature—what's worked in other models—and then test that in our model. We're interested in phytochemicals that are a significant part of our diet. We're not interested in studying phytochemicals we wouldn't normally consume dietarily. For example, right now we're focusing on indole-3-carbinol from cruciferous vegetables, such as broccoli, Brussels sprouts, and cabbage, and chlorophyll and its derivatives, which, of course, are present in all green leafy vegetables, especially spinach.

Q. Do you give a dose to the trout that is similar to what people might consume dietarily?

A. We typically start with very large doses just to see if we are going to see an effect at all. Then we can do a dose-response study to see what the degree of protection is at more realistic doses. For example, a few years ago we published a study with 10,000 trout in which we used six different concentrations of aflatoxin and six different concentrations of indole-3-carbinol.

With that number of fish, we could study levels of indole-3-carbinol that are quite similar to what a human would get from supplementation. Some of the phytochemicals we investigated are not only present in food but are also available over the counter as supplements. Indole-3-carbinol is one of the best-selling phytochemical supplements. We found a protective effect at levels of about 250 parts per million, which is fairly close to what the human dose would be with supplementation.

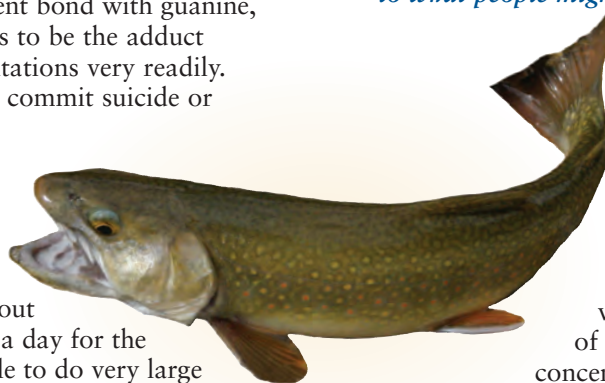
Q. Do you find that indole-3-carbinol and chlorophyll protect against cancer by the same molecular mechanism?

A. No, and that's what really makes this research particularly interesting. Indole-3-carbinol seems to work by a number of mechanisms. One involves the induction of enzymes that are responsible for detoxification of the carcinogen.

Q. Are those enzymes the same in trout and people?

A. They're very similar, and they respond to indole-3-carbinol in a similar manner. But there are other mechanisms, too. Scientists have shown that indole-3-carbinol can affect other important steps in the cancer process and cause programmed cell death, or apoptosis, where a cell that's been mutated commits suicide. There are sensors within the cell that sense that it's undergone mutations, and if it can't repair that DNA adduct or that mutation before it divides, then it programs itself to commit suicide.

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Q. Does chlorophyll protect by a different mechanism?

A. We're still studying that. Until fairly recently, it was thought that the sole mechanism of chlorophyll and its derivative, chlorophyllin, was binding to the carcinogen in the gut and preventing it from being absorbed. There was strong evidence for that. But now there's evidence for other mechanisms, too, because when we feed chlorophyll to trout, their livers turn green. So we know the chlorophyll is absorbed by the target tissue, suggesting that there could be mechanisms other than the binding mechanism.

Q. If these phytochemicals work by different mechanisms and against different carcinogens, would that suggest that it might be useful to combine indole-3-carbinol, chlorophyll, and other phytochemicals to try for more powerful prevention?

A. Yes, and that's a growing area in the chemoprevention field. If there are multiple mechanisms, then combining phytochemicals for an additive or synergistic effect would be a good strategy. For example, scientists have already shown that combining COX-2 inhibitors and phytochemicals has an additive effect in preventing colon cancer.

Q. Does cooking destroy phytochemicals in vegetables or alter them in a way that might reduce their cancer chemoprotective properties?

A. To some degree. It depends on the particular phytochemical. For example, with indole-3-carbinol, it's not such a problem because most of the derivatives we get from cooking are similar to the breakdown products you get after ingestion anyway. With chlorophyll or chlorophyllins, it's also not really a problem because some of the breakdown products in the body are similar to those formed from cooking. But there is some loss. As a general rule, I would say that these protective phytochemicals are probably higher in raw foods than in cooked foods.

Q. Once you get positive results from the trout experiments, what's the next step in assessing whether these phytochemicals may protect against cancer in humans?

A. We like to take a comparative approach, which we've done historically. If a mechanism is similar in fish, mice, and rats, it's likely that mechanism was conserved throughout evolution and probably works in humans as well.

Q. When people read about the protection afforded by phytochemicals in tea or vegetables, such as indole-3-carbinol, chlorophyll, or catechins, they're inclined to buy supplements that contain the purified extracts and take them in fairly high doses. What's the safety profile of these phytochemicals?

A. When people ask me that question I tell them it's best to get these protective phytochemicals from a balanced diet rather than from supplements. However, it may be difficult to get enough cruciferous vegetables in the diet. If you have an aversion to the taste of some of those foods, as I do, supplementation might be okay. The problem is that people tend to have the attitude that if a little is good for me, a lot must be even better, and that's not necessarily the case.

And you can take too much of some of these compounds. Sometimes the purified compounds don't have the same effect as the consumption of the whole foods. For example, for years epidemiological studies found a really good correlation between blood levels of beta-carotene and a decreased risk of lung and other cancers. Scientists in the chemoprotection field were convinced that beta-carotene had to be chemoprotective, but when they finally did an intervention study, it turned out that beta-carotene was not protective. In fact, it seemed actually to enhance slightly the risk of lung cancer in smokers. In that case, beta-carotene may have just been a marker for another chemical in those foods that was having a protective effect. We still don't know that for sure, but that's one example where we were fooled by the simple correlation-type studies.

Q. Are there any chemical differences between the phytochemicals that you give to the animals and phytochemicals in the diet? Are they the same chemical compounds?

A. Yes, they are.

Q. Some of your work shows that indole-3-carbinol given after exposure to a carcinogen may actually increase the risk of developing certain tumors in trout. How relevant do you think this is to people, because, of course, we would not know if we have cells that have already been initiated by a carcinogen?

A. That's an important question. We always try to design our experiments so that we can see both beneficial as well as adverse effects or toxicity. There are risks as well as benefits. We've done a number of studies showing that feeding indole-3-carbinol long-term after initiation—after exposure to the carcinogen—to trout and rats produced more liver cancer. In mice, though, there was less. Further investigation revealed that these disparate effects are related to sensitivity to estrogens. In other words, indole-3-carbinol may be acting as an estrogen. Genistein, a chemical in soy, also acts as a phytoestrogen.

Q. Does the phytochemical dose affect this cancer risk?

A. Based on the studies we've done, it would probably be very difficult to get a dose high enough to be a significant risk just from dietary cruciferous vegetables. However, if you eat cruciferous vegetables and supplement with a significant number of over-the-counter indole-3-carbinol tablets, and you plan to do that long term, there could be some risk. People who supplement should ask their doctor to check their liver function periodically because there could be some potential risk to the liver associated with long-term, high-dose supplementation. The problem with indole-3-carbinol is that it's a mixture of compounds. When you take an indole-3-carbinol tablet, it reacts with the acid in your stomach and very quickly forms a mixture of as many as 24 different compounds. We know very little about the potential toxicity of some of those individual compounds.

Q. What about the safety profile of chlorophyll or its synthetic derivative chlorophyllin?

A. That seems to be a different story. We've used chlorophyllin in human medicine for years. It's been used in geriatric patients as a pretty effective deodorant. In fact, I saw an Internet site where they were selling chlorophyllin tablets to deer hunters. They were marketing it as camouflage.

If you take the chlorophyllin tablets, you'll smell more like a plant and the deer won't be able to detect you. We haven't seen any adverse effects of high doses in humans, which made it really easy to move from animals to human application because chlorophyllin had already been used for other purposes in humans and found to be safe.

Q. Your recent research has focused on fetal exposure to carcinogens that may increase the risk of cancer in children. How did this hypothesis develop?

A. I became familiar with literature in cancer journals showing that a number of chemicals were capable of crossing the placenta and causing cancer in offspring born to mothers exposed to carcinogens. I got interested in the potential for altering the maternal diet and providing some protection for the fetus. If the chemical carcinogens cross the placenta, then protective phytochemicals may as well.

Q. What carcinogens commonly cross the placenta?

A. Probably the two that have been studied the most are polycyclic aromatic hydrocarbons, or PAHs, and arsenic. At least a dozen chemicals have been shown to be capable of crossing the placenta and causing cancer.

Q. What's the environmental source of arsenic? How is the mother exposed?

A. Arsenic is found in drinking water. Fortunately, in most places in the U.S., that's not much of a problem, but Artesian wells often have significant amounts of arsenic. In Bangladesh, the levels of arsenic in drinking water can be so high that there is a definite risk that babies born to those mothers will develop cancers.



Q. What's the source of the PAHs?

A. PAHs are found in the atmosphere. We breathe them all the time. They're also found in water, but the main exposure is through the diet. They get into plants, and they get into the animals that eat those plants. Even if you're a vegetarian, you're going to have some PAH exposure.

Q. Are PAHs concentrated in specific vegetables or fruits?

A. That's a good question. I haven't seen any research that has found that certain plants accumulate PAHs more than others. PAH exposure also depends on how you cook your food. Charcoal broiling typically enhances your exposure because when you burn that charcoal, the PAHs formed from combustion get deposited on the meat. And, of course, if you're a smoker, your exposure is increased from burning tobacco. One of the things that is really going to increase the exposure to PAHs in the coming years is coal burning in China. The global winds drift from west to east, so coal that burns in China gets in the atmosphere and then is deposited in the U.S., especially on the West Coast. Over the next 20 years there's going to be a huge boom in coal-fired energy production plants in China, so we may see a big increase in PAH exposure.

Q. Do some of these carcinogens also act as teratogens and cause birth defects?

A. That's not really known. Dioxins, which are chemically very similar to PAHs, do cause birth defects. For example,

dioxin causes cleft palate. So the carcinogens we study probably could cause birth defects, but we haven't found many relevant studies.

Q. How common are childhood cancers and what kinds of cancers do children develop?

A. The two most common types of cancer in children are leukemias and lymphomas, which are cancers of the blood, followed by brain tumors or cancers of the nervous system. Those are the major cancers in children, but cancer in children is not common. Cancer is the leading cause of death by disease in children up to the age of about 15. The most frequent cause of death in children is accidents, followed by cancer.

Q. How do you study this problem?

A. We use a mouse model. Pregnant mice are treated with an oral dose of the carcinogen about two or three days before they would give birth so there's ample time for the carcinogen to cross the placenta. Almost all of the offspring born to that mother develop a severe T-cell lymphoma and die between three and six months of age.

Q. Do the mothers develop cancer, too, as a result of exposure?

A. No. The adult seems to be relatively resistant, although we haven't followed the mothers for a long period of time. We've focused on the offspring. What's been really interesting to me is the dramatic effect of adding indole-3-carbinol to the mother's diet. The pups born to the mothers that were fed the indole-3-carbinol, even though the pups never got any indole-3-carbinol, are substantially protected against lymphoma.



Q. Are they protected because of enhanced detoxification of the carcinogen by liver enzymes?

A. That's one theory. We're not exactly sure of the mechanism. There might be other mechanisms at work, too.

Q. Have you looked at other phytochemicals using this model?

A. We are just finishing a study with tea. We used both regular green tea and decaffeinated green tea. We also tested a major phytochemical from green tea, epigallocatechin gallate (EGCG), alone or caffeine alone. So we had four different experimental groups. It was a little disappointing in that we didn't see much protection with tea. There was a hint of some protection with the caffeinated green tea, although it needs to be analyzed statistically. Caffeine alone did provide some protection. That could be important because the FDA specifically recommends that pregnant women cut down or refrain from caffeine ingestion. Those results are pertinent to lymphoma. In the surviving mice that don't get lymphoma, 100% of them get lung cancer. The EGCG alone seems to provide some protection against lung cancer, although we're having a statistician look closely at that. I'm not giving up completely on tea, but it doesn't

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look to be as effective as indole-3-carbinol from cruciferous vegetables in protecting against transplacental carcinogen exposure. We're just now starting a test with chlorophyll and chlorophyllin.

Q. Is lung cancer rare in children?

A. It is. In mice, it doesn't develop until they are about middle age. The number of children that get cancer is relatively low, but certainly cancers in middle-aged people and older are not rare, and lung cancer is still the #1 cause of death from cancer in males and females in the U.S. It would be exciting if this risk could be substantially reduced by phytochemical exposure during pregnancy. We're going to do some additional experiments to test that hypothesis.

Q. You also study ethnic differences in drug-metabolizing enzymes. Why is this important?

A. This field is known as pharmacogenetics. It's becoming increasingly important in medicine because the greatest number of hospitalizations are due to adverse drug reactions. A number of adverse drug reactions are due to genetic polymorphisms. Humans are not like inbred animals—we have a lot of genetic variability. Almost all of our enzymes involved in metabolizing drugs and carcinogens have what's known as genetic polymorphisms. In other words, there's more than a single form of the enzyme. There are sometimes many different possible forms of the enzyme, depending on how many mutations the individual has in the gene coding for the enzyme. These genetic polymorphisms are often related to ethnicity. That's certainly the case with the enzyme we've been studying, flavin-containing mono-oxygenase (FMO), because the expression seems to be confined to African-Americans and Hispanics, and we don't find the enzyme in Caucasians or Asians.

Q. In what tissue is the enzyme found?

A. It's found mostly in the lung. We've been trying to determine if that makes a difference for drugs that are toxic to the lung or metabolized by the lung. Inhalation is a very good drug delivery method. Some drugs just aren't very effective when you take them orally because they don't get absorbed very well. But the absorption is almost complete if the drug is inhaled. So it's a very effective method for drug delivery.

Q. Is that because of the vascularization of the lung tissue?

A. Yes. There's only a very small distance, about a micrometer, that a drug has to travel to be absorbed in the lungs, as opposed to how far it has to go through the lining of the GI tract. There's a huge surface area in the lung, and, again, it is highly vascularized. For all those reasons, drugs are absorbed very well from the lung. We've gotten some funding from pharmaceutical companies that are developing drugs for that purpose to look at whether FMO metabolizes certain drugs in the lungs.

Q. How are drugs metabolized?

A. Almost any chemical that gets into your body is metabolized before it's excreted. Metabolism helps to make the compound more water soluble and easier to excrete in urine or feces. Often, a hydroxyl group will get attached to a molecule to make it a little more water soluble. After that,

the compound may be sulfated as well. It becomes very water soluble because it's got an added polar group and a charge as a result of these chemical modifications.

Q. What drugs or chemicals have you been studying?

A. We have looked at compounds like thioureas, which are found in a number of chemicals and drugs. Lately, we've been studying ethionamide, which is a drug used to treat tuberculosis. Tuberculosis is a very significant health problem around the world that's increasing in incidence.

Q. Is the drug delivered by inhalation?

A. Yes. We want it to work in the lung. The drug is metabolized, and the sulfur group is oxygenated by bacterial enzymes, resulting in the death of the tuberculosis bacterium. We're interested in what happens if the human enzyme, instead of the bacterial enzyme, metabolizes that sulfur first. Does that lessen the effectiveness of the drug? My guess is that it probably does. There are other thiourea-containing compounds whose activation by this enzyme—by oxidizing that sulfur group—may make them toxic to the lung. That has happened in mice given a developmental drug for pain relief—the mice died from massive lung edema. We found out that this happened because the enzyme was metabolizing sulfur to a reactive sulfenic acid, which caused the toxicity.

Q. What percentage of African-Americans and Hispanics have the active FMO enzyme?

A. It turns out that all the Caucasians and Asians we have genotyped to date have a mutation that produces an inactive enzyme. About 27% of African-Americans and about 2-7% of Hispanics, depending on whether they're from Mexico or Puerto Rico, have at least one of the genes coding for an active enzyme. We predict that about 27% of African-Americans and 5% of Hispanics would metabolize these drugs differently because they have an active enzyme, whereas Caucasians and Asians do not.

Q. Are physicians aware of these ethnic differences so that they can calibrate the amount of a drug given to patients, according to their ethnicity?

A. Not in this particular case. Physicians are becoming increasingly aware of the importance of polymorphisms of some of the major drug-metabolizing enzymes. In the liver, for example, the major drug-metabolizing enzyme is cytochrome P4503A4, and physicians have learned that's an extremely important enzyme in the metabolism of drugs like cyclosporin. Cyclosporin is used in organ transplant patients to fight rejection by depressing the immune system. It's a tricky drug to work with because there's a very small difference between a therapeutic dose and a toxic dose. It's metabolized mainly by cytochrome P4503A4, and the amount of this enzyme in the liver can vary among individuals by ten- to twenty-fold. That can make a big difference in the drug dosage.

Q. Are some of these liver enzyme polymorphisms also related to ethnicity?

A. Yes, almost all of the genetic polymorphisms in these drug-metabolizing enzymes have an ethnic difference. It's almost the rule. Within 10 to 20 years it will be pretty typical to measure the level of these enzymes before setting drug dosage. That will avoid a lot of the adverse drug-drug interactions that cause so many people to end up in the hospital because of an overdose—they may be deficient in

an enzyme that metabolizes the drug so they can't get rid of it as fast as a person of different ethnicity.

Q. *Could there be ethnic differences in the way indole-3-carbinol protects against certain cancers?*

A. Yes. That's actually an under-studied area. If we could use this knowledge in medicine to design the proper dose of a therapeutic drug for an individual, then we could do the same thing with phytochemicals for protection against diseases like cancer. For example, we could genotype people once we know the mechanism by which the phytochemical works. Then we could figure out what genes are important in that mechanism and test individuals for their enzyme activity. That would allow us to better determine the optimum intake of certain phytochemicals.

Q. *What dietary strategies do you think people might consider in order to minimize the risk of developing cancer?*

A. I think the recommendation that has been advocated by a number of agencies like the National Cancer Institute and the Association for Cancer Research to consume five to nine servings of fruits and vegetables a day is still a good rule of thumb. Most of the really effective phytochemicals against cancer tend to be in vegetables rather than in fruit. Cutting down consumption of meat and eating more fish is also good. If you're a woman of child-bearing age, you have to choose fish carefully to avoid mercury. Food should be prepared in ways to minimize or eliminate polycyclic aromatic hydrocarbons and cooked-meat mutagens. Aside from that, I recommend some daily supplements that I take myself, such as lipoic acid, a multi-vitamin pill, 500 mg of vitamin C, and 400 IU of natural vitamin E. I think that supplements are valuable in addition to a whole food diet.

Q. *Aflatoxin exposure doesn't seem to be a prevalent public health problem in this country. Do you feel there is any risk from eating peanuts, peanut butter, or bread?*

A. It really isn't much of a health problem in the U.S. The FDA watches it pretty closely and their action level for aflatoxin in food products is 20 parts per billion, which rarely happens. I think the models that the FDA used to estimate cancer risk from aflatoxin are pretty conservative. There is actually much less liver cancer than would be predicted from aflatoxin exposure. I think we're pretty safe in the U.S. with respect to aflatoxin. That's not true in parts of China where mold grows on grain because it's hot and humid.

Q. *Do you think that some dietary phytochemicals may be useful in treating cancer?*

A. That's hard to know. One of the driving forces for drug development in the U.S. is money. Obviously you can't make a lot of money studying compounds like phytochemicals that can't be patented, so therapeutic phytochemical research won't proceed as quickly as drug research. It's becoming a focus for researchers, but it's very difficult.

Q. *Have you used trout or small rodents to study the potential therapeutic use of phytochemicals?*

A. We haven't done that in trout because it takes too long for the tumor to get big enough to evaluate regression, but an athymic or nude mouse model can be used. In these mice, the thymus is removed and their immune system is completely compromised. You can implant a human tumor cell in that mouse, and the tumor will grow as it does in a human. For example, you can take human breast cancer cells and inject them under the skin of a mouse. You can then feed the mouse different phytochemicals to see if that tumor shrinks or its growth is inhibited. I'm planning to do a study with human cancer cells and indole-3-carbinol to see if we can get tumor regression. We can inhibit lymphoma transplacentally, but now we want to know if indole-3-carbinol will inhibit its growth once it's formed. So we will be continuing our cancer chemoprotection studies and also investigating potential therapeutic roles. **LPI**



Cancer Prevention by Chlorophylls

Michael T. Simonich, Ph.D.
LPI Research Associate

Numerous chemicals from fruits and vegetables protect against the damage wrought by carcinogens in experimental animal models. Usually these chemicals occur in edible plants at such low levels that doses sufficient for protection are not practically attained even in a balanced diet. Because of their abundance in green vegetables, chlorophyll and its widely used derivative, chlorophyllin, have attracted attention as potential anti-carcinogens. Scientists in our laboratory have studied the chemoprotective effects of chlorophyllin and have recently discovered that natural chlorophyll itself is a potent anticancer agent.

Chlorophyllin is easily and inexpensively made from crude chlorophyll and has been used for decades without known human toxicity as a food dye, a wound-healing accelerant, and for odor control. The anticancer properties of chlorophyllin have been extensively reported in dozens

of studies from cell culture to rats, where different chemical carcinogens were used to initiate cancer. From these studies we have learned that chlorophyllin acts primarily as a blocking agent against chemical initiation of carcinogenesis. Simply put, chlorophyllin is most effective when administered along with the carcinogen, thereby blocking cancer-initiating activity. Molecular complexes formed between chlorophyllin and carcinogen molecules are physically too large to be absorbed from the gut. Because fewer carcinogen molecules reach the target organ, less DNA damage occurs, and the chance of tumor development is diminished. Consistent with the blocking mechanism, chlorophyllin is generally much less effective if administered after the carcinogen, i.e., once the carcinogen's damage has been done.

Importantly, protection by chlorophyllin extends beyond a single chemical carcinogen to include aflatoxins from heavy fungal contamination of grain or nuts; heterocyclic amines, whose primary source of exposure is overcooked meat; and polycyclic aromatic hydrocarbons (PAH), an increasingly abundant class of combustion-derived air pollutants. While aflatoxin B₁ exposure is insignificant in

continued on page 10

DIET AND OPTIMUM HEALTH

A CONFERENCE ORGANIZED BY THE LINUS PAULING INSTITUTE
CO-SPONSORED BY THE OXYGEN CLUB OF CALIFORNIA AND OREGON HEALTH & SCIENCE UNIVERSITY

MAY 16-19, 2007 • HILTON HOTEL, PORTLAND, OREGON

CONFERENCE TOPICS

Health promotion and disease prevention by lifestyle and diet, including vitamins, minerals, and phytochemicals; and the role of oxidative stress and antioxidants in human health and disease

Wednesday, May 16, 2007

- 2:00 PM Registration begins
5:00 - 7:00 **LPI Young Investigator Forum**
7:00 Welcome Reception

Thursday, May 17, 2007

- 6:00 AM Organized Walk/Run
7:30 Breakfast
8:15 Welcome and Introduction
*Maret G. Traber, Linus Pauling Institute,
Oregon State University, Corvallis, OR*

FLAVONOID FUNCTIONS

- 8:30 - 9:00 Overview of Flavonoids in Human Health
*Gary Williamson, Nestlé Research Center,
Lausanne, Switzerland*
9:00 - 9:30 Flavonoid Metabolism and Disposition
*Augustine Scalbert, INRA Centre de Clermont-
Ferrand/Theix, Saint-Genes-Champagnelle, France*
9:30 - 10:00 Isoflavones: Benefit or Harm?
*William Helferich, University of Illinois at
Champaign-Urbana, Urbana, IL*
10:00 - 10:30 Coffee/Tea Break

NEW DISCOVERIES OF VITAMIN C IN HEALTH AND DISEASE

- 10:30 - 11:00 Ascorbylation Reactions
*Fred Stevens, Linus Pauling Institute,
Oregon State University, Corvallis, OR*
11:00 - 11:30 Intravenous Vitamin C and Cancer Therapy
*Mark Levine, National Institute of Diabetes and
Digestive and Kidney Diseases, Bethesda, MD*
11:30 - 12:00 PM Ascorbic Acid Transporters in Health
and Disease
*Matthias Hediger, Institute of Biochemistry and
Molecular Medicine, Berne, Switzerland*
12:00 - 1:30 Lunch

LIPOIC ACID: BIOLOGICAL MECHANISMS OF ACTION

- 1:30 - 2:00 Lipoic Acid as an "Anti-aging" and Anti-
inflammatory Agent
*Tory Hagen, Linus Pauling Institute,
Oregon State University, Corvallis, OR*
2:00 - 2:30 Exercise Training and the Antioxidant
Alpha-Lipoic Acid in the Treatment of Insulin
Resistance and Type 2 Diabetes
*Erik Henriksen, University of Arizona
College of Medicine, Tucson, AZ*
2:30 - 3:00 Mechanism of Lipoic Acid Action in
Multiple Sclerosis
*Dennis Bourdette, Oregon Health & Science University,
Portland, OR*
3:00 - 3:30 Coffee/Tea Break

NEUROPROTECTION BY ANTIOXIDANTS

- 3:30 - 4:00 Deficiency of Vitamins E and C Causes Severe
Central Nervous System Damage
Ray Burk, Vanderbilt University, Nashville, TN
4:00 - 4:30 Neuroprotective Properties of the Natural
Vitamin E Alpha-Tocotrienol
*Chandan Sen, The Ohio State University
Medical Center, Columbus, OH*
4:30 - 5:00 Dietary Antioxidant Intakes and Neurologic
Disease Risk
*Alberto Ascherio, Harvard School of Public Health,
Boston, MA*
5:00 - 7:00 **Poster Session**

Friday, May 18, 2007

- 6:00 AM Organized Walk/Run
7:30 Breakfast

MATERNAL DIET, GENES, AND EPIGENETICS

- 8:30 - 9:00 Cancer and Imprinting: Early Nutritional
Influences
*Randy Jirtle, Duke University Medical Center,
Durham, NC*

- 9:00 - 9:30 Indole-3-Carbinol in the Maternal Diet of Mice Acts as a Transplacental Cancer Chemoprotection Agent
David Williams, Linus Pauling Institute, Oregon State University, Corvallis, OR
- 9:30 - 10:00 Choline: Critical Role During Fetal Development
Steven Zeisel, University of North Carolina at Chapel Hill, NC
- 10:00 - 10:30 Coffee/Tea Break

CANCER CHEMOPREVENTION

- 10:30 - 11:00 Histone Deacetylase Inhibitors in Cancer
Emily Ho, Linus Pauling Institute, Oregon State University, Corvallis, OR
- 11:00 - 11:30 Nutrition and Cancer Prevention
Johanna Lampe, Fred Hutchinson Cancer Research Center, Seattle, WA
- 11:30 - 12:00 PM Redox-Sensitive Transcription Factors as Prime Targets for Chemoprevention with Anti-inflammatory and Antioxidative Phytochemicals
Young-Joon Surh, Seoul National University, Seoul, South Korea
- 12:00 - 1:30 Lunch

OBESITY, EXERCISE, AND GENE REGULATION

- 1:30 - 2:00 Exercise Neuroscience
Jacque Van Hoomissen, University of Georgia, Athens, GA
- 2:00 - 2:30 Control of Energy Homeostasis: Role of Enzymes and Intermediates of Fatty Acid Metabolism in the Central Nervous System
Dan Lane, Johns Hopkins University School of Medicine, Baltimore, MD

- 2:30 - 3:00 Calorie Restriction Mimetics
Don Ingram, National Institute on Aging, Intramural Research Program, Baltimore, MD
- 3:00 - 3:30 Carbohydrate Restriction Alters Lipoprotein Metabolism During Weight Loss
Maria-Luz Fernandez, University of Connecticut, Storrs, CT
- 3:30 - 4:00 Coffee/Tea Break

AWARD CEREMONY FOR LINUS PAULING INSTITUTE PRIZE FOR HEALTH RESEARCH

Chair: Balz Frei, Linus Pauling Institute, Oregon State University, Corvallis, OR

- 4:00 - 5:30 Plenary Lecture by Awardee
- 6:30 Reception
- 7:00 Banquet

Saturday, May 19, 2007

- 7:30 AM Breakfast

PUBLIC SESSION

Chair: Maret G. Traber, Linus Pauling Institute, Oregon State University, Corvallis, OR

- 9:00 - 11:00 Secrets of the Lean Plate Club
Sally Squires, The Washington Post, Washington, DC

For more information about the Conference, please see the LPI Web site at <http://lpi.oregonstate.edu> or phone the Institute at 541-737-5075. Registration information is available starting in November.



Linus Pauling Legacy Award

Dr. John Roberts, Professor of Chemistry Emeritus, California Institute of Technology, was the fourth recipient of the Linus Pauling Legacy Award on May 4, 2006. The award was established in 2001 and dedicated to the recognition of outstanding achievement by an individual or organization in a subject of interest to Linus Pauling (1901-1994). Previous Awards went to Daisaku Ikeda, Sir Joseph Rotblat, and Dr. Matthew Meselson.

Dr. Roberts was honored “for his pioneering and creative contributions to the study of chemistry, including major breakthroughs in the scientific understanding of reaction mechanisms, fundamental cross-disciplinary applications of physical chemistry to organic chemistry, and momentous advancements in the techniques of nuclear magnetic resonance spectroscopy; and for his decades of leadership as a voice for, and example of, uncompromising ethics and

integrity in scientific research.” Pauling and Roberts first met in 1947. A few years later, Roberts joined the Caltech chemistry department, where Pauling was Chairman. In 1956, Pauling said, “I think that Professor Roberts is one of the most promising, able, and original organic chemists in the country.” Indeed, Roberts’ contributions to organic chemistry have been described as fundamental, and he has been honored with the Priestley Medal, the National Medal of Science, and many other awards. Roberts’ Legacy Award Lecture, based on some of his developmental research, was entitled, “Useful Knowledge about NMR [nuclear magnetic resonance] and MRI [magnetic resonance imaging]—How They Work and How They are Used.”

For more information, please see the Spring 2006 issue of *The Messenger*, published by OSU Libraries (special.collections@oregonstate.edu).

developed countries with safe grain storage methods, it is prevalent in Asia and sub-Saharan Africa, where it contributes to astonishingly high rates of liver cancer. The latter two carcinogen classes represent significant exposures for the U.S. population, given high consumption of grilled meats and huge increases in airborne PAH-bound particulate matter, especially from diesel exhaust.

Significant protection against cancer by chlorophyllin was reported in the Fall/Winter 2002 *LPI Research Newsletter* ("Chlorophylls and Cancer Prevention: Passing the First Hurdle," by Dr. George S. Bailey). That article summarized the results of a clinical trial of dietary chlorophyllin supplementation in a human population in eastern China with chronic, unavoidably high aflatoxin exposure and a high incidence of liver cancer. Administration of 100 mg of dietary chlorophyllin (in pill form) thrice daily led to a highly significant 55% reduction in the amount of aflatoxin-DNA adducts (substances connected by a chemical bond; in this case, indicative of DNA damage) in the urine of participants. Elevated urinary output of this hepatic DNA adduct biomarker in humans is clearly associated with increased risk of liver cancer, and diminished levels of aflatoxin-DNA adduct are associated with reduced liver cancer risk in several animal studies. Thus, simple dietary supplementation with chlorophyllin might cut human liver cancer risk in half for people chronically exposed to high levels of dietary aflatoxin. A long-term, 20-year clinical trial is now being conducted by Chinese investigators to evaluate the reduction of liver cancer incidence by chlorophyllin.

In impoverished regions, where diet choices are limited to survival staples and grain storage methods and pollution exposure will only improve with increases in the standard of living, dietary supplementation with chlorophyllin might be the easiest and most effective protection strategy to implement. In the developed world, a diet high in natural chlorophyll from vegetable consumption could offer substantial protection against food- and air-borne carcinogens, in addition to all the other known benefits of a vegetable-rich diet.

Dietary chlorophyll intake comparable to the 300 mg per day of chlorophyllin administered in the Chinese human intervention trial is obtainable by moderate-to-high consumption of green vegetables. Chlorophyll has no known human toxicity, but its protective properties have been little studied. This is likely due to the extraordinary cost of commercially pure chlorophyll (necessary for unambiguous experimental evaluation), or the difficulty and expense of purification in the laboratory.

Chlorophyll is potentially anti-mutagenic and was recently shown to induce carcinogen-detoxifying enzymes of phase 2 metabolism in cell cultures. A few studies have examined natural chlorophyll as a cancer preventative in animals. In rainbow trout, exposure to 200 parts per million (ppm)

of dibenzo[*a,l*]pyrene (the most carcinogenic PAH known) resulted in hepatic DNA-adduct formation. Adduct formation was reduced 66% by co-exposure to 3000 ppm chlorophyll in the diet, which was nearly identical to the protection we observed by a similar dietary co-exposure to chlorophyllin. Another lab reported that dietary spinach or an equivalent dose of chlorophyll equally inhibited the proliferation of colon cells in rats induced by heme, an iron-containing pro-oxidant from red meat that is correlated with increased risk of colon cancer. The chlorophyll-containing diets also largely blocked formation of a toxic heme metabolite. The authors speculated that green vegetables may decrease colon cancer risk from dietary heme through the protective effects of chlorophyll.

Our lab's current research is focused on the rigorous testing of cancer prevention by natural chlorophyll in trout and rats. We recently examined protection by dietary chlorophyll in a rainbow trout multi-organ tumor model. Duplicate groups of 140 juvenile trout were exposed via the diet for four weeks to 224 ppm dibenzo[*a,l*]pyrene (DBP) alone, or with 1000, 2000, 4000, or 6000 ppm chlorophyll, then returned to the control diet. DBP induced high tumor incidence (number of fish with at least one tumor in each treatment group) in the liver (51%) and stomach (56%), and a low incidence in swim bladder (10%) nine months after initiation. Co-feeding 2000, 4000, or 6000 ppm chlorophyll significantly reduced stomach tumor incidence to 29, 23, and 19%, respectively, and liver tumor incidence to 21, 28, and 26%, respectively. A troubling result from the study was that dietary chlorophyllin given after carcinogen exposure (DBP diet, followed by 2000 ppm chlorophyllin for the duration of the study) had no effect on liver or stomach tumor response but promoted swimbladder tumor incidence to 38%. This finding supports previous evidence that chlorophyllin chemoprevention is not without some potential risk. Post-initiation promotion by chlorophyllin has previously been reported in the rat colon in specific experimental designs. However, in the one study where post-initiation effects of natural chlorophyll on colon carcinogenesis were examined, chlorophyll suppressed rather than promoted pre-cancerous lesions of the rat colon. The available evidence from previous studies and our recent finding in trout suggest that natural chlorophyll may be superior to chlorophyllin as a choice for chemoprevention in humans.

Our finding that chlorophyll substantially reduced tumor formation in trout compelled us to look for similar protection by chlorophyll in rats. We first studied protection against early biomarkers of carcinogenesis. Three groups of seven rats each received five daily doses of 250 ppb aflatoxin B₁ alone, aflatoxin with 250 ppm chlorophyllin, or aflatoxin with natural chlorophyll equivalent to 250 ppm chlorophyllin. The aflatoxin was attached to a radioactive isotope, or radiolabeled, so that we could follow and measure it. Chlorophyllin and chlorophyll strongly reduced liver DNA adduction by 42% and 55%, respectively, and serum albumin adducts by 65% and 71%, respectively. The feces of chlorophyllin- and chlorophyll-treated rats contained 137% and 412% more radiolabel, respectively, than control (aflatoxin only) feces, indicating that chlorophyllin and chlorophyll inhibited aflatoxin uptake from the gut, restricting its distribution to the GI tract. This finding is consistent with chlorophyllin acting as a blocking agent, i.e., by binding





Vitamin E Pharmacokinetics and Oxidative Biomarkers in Normal and Obese Women

Maret G. Traber, Ph.D., LPI Principal Investigator, Professor of Nutrition

In Spring 2006 our lab got some exciting news. The NIH had requested that intramural investigators collaborate

with scientists outside NIH and propose studies for the “Bench to Bedside” grant program. Mark A. Levine of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) and I wrote a proposal to study vitamin E requirements in women. It was announced in June 2006 that our proposal was one of just 19 proposals selected for funding. The “Bench to Bedside” program is designed to speed translation of promising laboratory discoveries into new medical treatments. The Traber-Levine project will conduct research related to women’s health. Additional personnel at NIDDK who will work on this project include Sebastian J. Padayatty, He Sun, and Robert Wesley. Fred Stevens and Scott Leonard of LPI will also participate.

The rationale for our proposal is that although vitamin E (α -tocopherol) is essential for humans, there is no known “specific” α -tocopherol function, and thus it is difficult to set human dietary requirements. The current α -tocopherol requirements are based on studies in the 1950s and 60s in vitamin E-depleted men—psychiatric hospital patients—fed rancid fat for five years. These studies estimated the average daily requirement of vitamin E to be 12 mg of α -tocopherol. It is difficult for most Americans to obtain this much vitamin E in their usual diets. Vitamin E is present in nuts, seeds, and vegetable oils, such as sunflower oil, safflower oil, and olive oil. One cup of cooked spinach has about 6 mg; it would take more than two cups to get the current Recommended Dietary Allowance of 15 mg. Since less than 10% of Americans meet the vitamin E requirement, there is a question as to whether 12 mg of α -tocopherol per day is a valid estimate. Importantly, women were not studied, so there are no data available on women’s requirements.

We plan to study healthy lean women, obese women, and diabetic obese women to estimate α -tocopherol pharmacokinetics and establish some additional relevant biomarkers. These data will be used to predict α -tocopherol requirements and to set new recommendations for vitamin E intakes. An accurate estimate of human dietary α -tocopherol requirements will ensure that adequate antioxidant protection can be obtained without excessive fat consumption. This concept is especially important for obese women who are advised to consume less fat and thus eat fewer vitamin E-containing foods.

Our hypothesis is that tissue stores of α -tocopherol are critical to its antioxidant function and that α -tocopherol delivery to tissues can be calculated from plasma α -tocopherol turnover. α -Tocopherol turnover will be characterized by the simultaneous oral and intravenous administration of two differently isotope-labeled α -tocopherols to the subjects. We will then calculate vitamin E absorption and disappearance rates in the different groups. Because we demonstrated earlier this year that vitamin C recycles vitamin E in cigarette smokers that have high oxidative stress, we will determine if vitamin C status is important for vitamin E status. Subjects will be studied twice: first, after they have consumed a “no” vitamin C diet for about four weeks until they have almost no measureable vitamin C in their plasma, and again after they have consumed vitamin C supplements for a month. These studies will directly test in the same subjects if vitamin C intakes affect vitamin E status in normal subjects. We also plan to conduct two pilot studies to determine the optimal fat intake for vitamin E absorption and the size of the vitamin E dose that does not alter vitamin E kinetics.

We are especially excited to be chosen for this special NIH project and hope to report our findings in two years!

LPI

the carcinogen and blocking its uptake to the bloodstream. A similar result for chlorophyll suggests that it, too, may protect by this mechanism. We examined whether chlorophyllin and chlorophyll protection might also occur at the metabolic level, after uptake from the gut. Chlorophyllin and chlorophyll were both recently shown by some of our collaborators to induce higher activity levels of the carcinogen-detoxifying enzymes of phase 2 metabolism in cell cultures. However, in the rat liver, neither co-treatment induced activity of the phase 2 enzymes quinone reductase and glutathione S-transferase (GST) above control levels.

A second study was done to examine the protection against late pathophysiological markers in the rat liver and colon. We specifically examined the effect of chlorophyllin and chlorophyll co-treatment on GST-placental form positive staining foci in the liver and aberrant crypt foci in the colon. Both foci types are small populations of cells that have undergone a hyperplastic transformation and, while not yet cancerous, will often become so. Twenty-nine rats in three experimental groups were treated with chlorophyll or chlorophyllin for ten days. At 18 weeks after carcinogen exposure, the chlorophyllin and chlorophyll co-treatments had reduced

the percentage of GST-placental form positive foci in the liver by 74% and 77%, respectively, compared to control livers. Chlorophyllin and chlorophyll reduced the mean number of aberrant crypt foci per colon by 63% and 75%, respectively.

Our results show that both chlorophyllin and chlorophyll protect against early biochemical and late pathophysiological biomarkers of aflatoxin carcinogenesis in the rat liver and colon, and against PAH-initiated cancer in the rainbow trout. These studies provide the first demonstration in any animal model of cancer chemoprotection by dietary natural chlorophyll, which may be a less problematic choice for human intervention than its derivative chlorophyllin. While our results are entirely consistent with chlorophyll acting as a blocking agent like chlorophyllin, further experiments are needed to determine the precise inhibitory mechanism by chlorophyll of aflatoxin and dibenzo[*a,l*]pyrene uptake. The results also support the idea that increased consumption of vegetables with high chlorophyll content may substantially lower cancer rates among human populations at high risk from exposure to aflatoxin and polycyclic aromatic hydrocarbons.

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The Beverage Guidelines: What Should You Drink to Stay Healthy?

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

Why Do We Need Beverage Guidelines?

The Beverage Guidelines were developed by Drs. Barry Popkin, Lawrence Armstrong, George Bray, Benjamin Caballero, Walter Willett, and me, and published this year in *The American Journal of Clinical Nutrition* ("A new proposed guidance system for beverage consumption in the United States," *Am. J. Clin. Nutr.* 83: 529-42, 2006). The Beverage Guidance Panel was assembled to provide guidance on the relative health and nutritional benefits and potential health risks of various beverage categories by systematically reviewing the relevant scientific literature.

We undertook this project to understand the effects of beverages on total energy intake. (Note that "energy" is used synonymously with "calories" throughout this article.) We were also concerned about the increased use of sugar in the global diet. For instance, in the United States the average adult consumes over 21% of total calories from beverages—more than double what it was several decades ago. Between 1977 and 2001, the proportion of energy obtained from calorically sweetened soft drinks and fruit drinks (which are different from fruit juices) has increased from 3.9% to 7.1% of all daily calories (from 70 to 189 kcals per day) for the average American aged two and older. These trends are echoed in many countries, where sugar intake from many different sweetener sources has increased considerably in the past 30 years, much of which has come from beverages.

The Beverage Guidance System

Our panel ranked water as the preferred beverage to fulfill daily fluid needs for adults, followed by tea and coffee, low-fat (1.5% or 1%) and skim (nonfat) milk and soy beverages, noncalorically sweetened beverages, beverages with some nutritional benefits (fruit and vegetable juices, whole milk, alcohol, and sports drinks), and—with the lowest priority—calorically sweetened, nutrient-poor beverages.

In coming up with this rank order, we considered the following characteristics of beverages:

- Energy and nutrient density
- Contribution to total energy intake and body weight
- Contribution to daily intake of essential nutrients
- Evidence for beneficial health effects
- Evidence for adverse health effects

Based on this rationale, different combinations of beverages can be used to fulfill the fluid needs of a healthy person.

Water

Water consumption is necessary for normal metabolism and physiological function and may provide essential minerals, such as calcium, magnesium, and fluoride. Potable water is very safe and has no adverse health effects. Acute dehydration results in impaired cognition, moodiness, poor thermoregulation, reduced cardiovascular function, and impaired physical work capacity. In general, excess water intake does not cause adverse effects in healthy individuals with properly functioning kidneys because the kidneys can produce a large volume of urine in a relatively short period of time to correct any disturbance.

Tea and Coffee

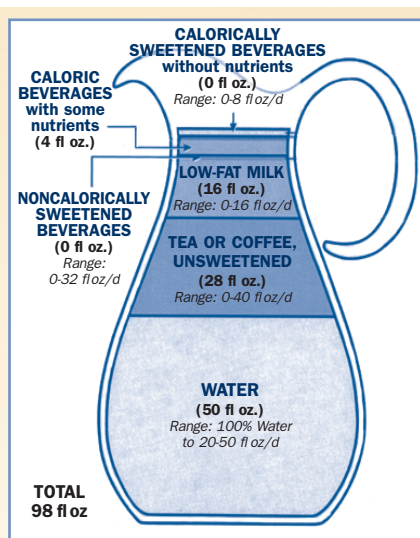
Black, green, and oolong teas are the three main categories of tea consumed in the world. Tea provides a variety of flavonoids and antioxidants, as well as a few micronutrients, such as fluoride. While there is solid evidence that tea protects against chemically induced cancers in experimental animals, it remains unclear whether tea consumption lowers cancer risk in humans. Tea also provides some amino acids, mainly L-theanine, which has been claimed to cause a relaxed, yet

alert state of mind and improve immunity. Tea consumption may also increase bone density, reduce tooth decay and cavities, and lower formation of kidney stones. In addition, epidemiological studies suggest that daily consumption of at least three cups of tea is associated with a modest decrease in the risk of myocardial infarction (heart attack). Addition of sugar, milk, or cream to tea and coffee would make these beverages less desirable due to increased energy and fat content.

With respect to coffee consumption, several studies have observed significant inverse associations with the risk of type 2 diabetes. A modest inverse association between decaffeinated coffee consumption and risk of type 2 diabetes has also been

observed, suggesting that compounds other than caffeine may contribute to risk reduction. High intakes of coffee have been associated with significant reductions in colorectal cancer risk in many studies, but others have not found such significant associations. Coffee and caffeine consumption have been consistently associated with significant reductions in the risk of Parkinson's disease in men but not women, which may be due to the modifying effects of estrogen. Boiled, unfiltered coffee, in contrast to normal filtered coffee, has been found to have adverse cardiovascular effects due to increased serum cholesterol.

There is more caffeine in coffee than in tea. Although caffeine is a mild diuretic, human studies indicate that caffeine consumption of up to about 500 milligrams per day does not cause dehydration. A caffeinated beverage's fluid content compensates for its acute diuretic effect. To date, the evidence suggests that moderate caffeine intake up to 400 milligrams per day is not associated with increased risk of heart disease, hypertension, osteoporosis, or high cholesterol. Pregnancy and aging may affect an individual's sensitivity to caffeine. Pregnant women are often advised to limit caffeine consumption because caffeine intakes higher than 300 milligrams per day have been associated with increased risk of miscarriage and low birth weight.



Some studies indicate that the magnitude of caffeine's effect is smaller at low and high intake levels but greater at intermediate levels. Such a relationship has been reported for exercise performance time, reaction time, vigilance, information processing, and mood but may not exist for all physiological and psychological responses.

Low-fat (1.5% or 1%) and Skim (Nonfat) Milk and Fortified Soy Beverages

For children, milk is currently the main source of vitamin D and calcium. Milk is also an excellent source of high-quality protein. Low-fat and skim milk, including low-fat yogurt drinks, can contribute to a healthy diet but are not essential for it. Fortified soy milk is a good alternative for individuals who prefer not to consume cow's milk, although soy milk cannot be legally fortified with vitamin D in the United States and some other countries. Soy milk also is a good source of calcium, providing about 75% of the calcium bioavailable from milk. Yogurt drinks have lower lactose content than milk and may be preferred by individuals with reduced lactose tolerance. In general, low-fat dairy beverages and fortified soy milk are important sources of protein, calcium, and other essential micronutrients.

A number of beneficial and some detrimental health effects have been attributed to the consumption of cow's milk. At present, there is insufficient scientific evidence to support an effect of milk or dairy products on weight loss, despite heavy advertising by the dairy industry. Milk may improve bone health; however, the benefits of higher calcium intake on bone mineral density are not maintained if the high intake is reduced. Milk is an important source of calcium and vitamin D due to fortification, particularly for children and adolescents. Milk products also contribute to the intake of essential nutrients in children and adolescents. There is, however, some evidence that milk may increase the risk of aggressive prostate cancer and possibly some other cancers, due to the presence of insulin-like growth factor.

Noncalorically Sweetened Beverages

The noncalorically sweetened beverages (diet sodas and other "diet" drinks) are preferable to calorically sweetened beverages (regular sodas) because they provide water and sweetness but no calories. There is no scientific evidence that noncaloric sweeteners like aspartame increase cancer risk in humans. Evidence is emerging suggesting that the high sweetness of noncalorically sweetened beverages may contribute to a conditioning for a high preference for sweetness. Thus, these beverages are less desirable than water, tea, or coffee.

Caloric Beverages with Some Nutrients

Fruit juices (100% juice) provide most of the nutrients of their natural source but are also relatively high in energy content and may lack fiber and other beneficial compounds present in the whole fruit. Vegetable juices, such as tomato and multi-vegetable juices, are a healthy alternative to fruit juices. They have fewer calories than orange or other fruit juices but usually have significant amounts of added sodium, which is a risk factor for hypertension.

Whole (full-fat) milk contains a large amount of calories and excessive saturated fat and should be avoided. The adverse effects of saturated fats on the risk of cardiovascular diseases are well documented.

Sports drinks contain 50 to 90% of the energy in calorically sweetened soft drinks and provide small amounts of sodium, chloride, and potassium. They help athletes during high endurance activities but should be consumed sparingly, except for endurance athletes, because they provide "hidden" calories.

For adults, alcoholic beverages consumed in moderation can have health benefits, such as a significantly decreased risk of coronary heart disease and ischemic stroke, and possibly a reduced risk of type 2 diabetes. Moderate intake is defined as the daily consumption of no more than one drink for women and two for men. (One drink is 12 fl oz of beer, 5 fl oz of wine, or 1.5 fl oz of distilled spirits.) Alcoholic beverages contain calories, and excessive alcohol consumption is linked to serious health and social problems. The major health benefits of alcoholic beverages are attributed to alcohol per se, which increases serum levels of high-density lipoprotein (the "good" cholesterol). Current evidence suggests some additional health benefits of flavonoids in red wine or dark beer.

Calorically Sweetened Beverages

Calorically sweetened beverages with high energy density and very limited amounts of other nutrients are not recommended and should be consumed sparingly or not at all. These include carbonated and non-carbonated beverages, such as regular sodas and fruit drinks, usually sweetened with high-fructose corn syrup or sucrose. These caloric sweeteners, which provide hidden calories in beverages, have been linked to dental caries, excess energy intake, weight gain, and type 2 diabetes.

How Much of Each Beverage Should You Consume?

Most adults with an energy requirement of 2,200 kcal per day need to drink about 100 fl oz (3 liters) of beverages. At most, 10% of the daily total calories should come from beverages. Based on this reasoning, we generally recommend the following daily amounts of the different beverages:

- Water, 20-50 fl oz (but can be up to the full daily beverage intake of about 100 fl oz)
- Tea and coffee (unsweetened, no or little milk or cream added), 0-40 fl oz
- Low-fat or skim milk and soy beverages, 0-16 fl oz
- Noncalorically sweetened beverages, 0-32 fl oz
- Caloric beverages with some nutrients: 100% fruit or vegetable juices, 0-8 fl oz; whole milk, 0 fl oz; sports drinks, 0-8 fl oz; alcoholic beverages, 0-1 drink for women and 0-2 drinks for men
- Calorically sweetened beverages, 0-8 fl oz

What Can the Food Industry and Government Do?

While self-policing in the beverage industry is unlikely, manufacturers can reduce calories in all beverages. To encourage more healthful choices, government could tax added sugar in all beverages so that the relative cost of most calorically sweetened beverages increases greatly. However, it is not known if this would achieve the desired result of reducing energy intake. Reducing or eliminating advertisements for these products would also help, especially to young children and adolescents, as would health education programs in schools. **LPI**



Conjugated Linoleic Acid-rich Chicken Eggs as a Functional Food

Gita Cherian, Ph.D.
Associate Professor
OSU Department of Animal Sciences

Cardiovascular disease (CVD) continues to be the principal cause of death in the United States. Because the current U.S. diet is rich in saturated fatty acids, cholesterol, and linoleic acid, it is proatherogenic, prothrombotic, proinflammatory, and proaggregatory, leading to CVD. A diet-based approach offers great potential to reduce CVD, as about 80% of heart attacks can be avoided by diet modification and lifestyle changes. Conjugated linoleic acids (CLA) are isomers of linoleic acid (a polyunsaturated omega-6 fatty acid) that have the same chemical composition but different spatial orientations that affect their physiological roles. The major isomer is the *cis*-9, *trans*-11 form. CLA have received considerable attention for their antiatherogenic, anticancer, and body fat-reduction properties. Dietary CLA is contributed by ruminant foods, such as dairy and beef. Based on animal data, it is estimated that approximately three grams per day of CLA would be required to produce beneficial effects in humans, but the current consumption of CLA is less than 600 milligrams per day. As Americans opt for low-fat dairy products and choose more poultry than beef, it is possible that the dietary supply of CLA from ruminant foods will be further limited. Chicken eggs, due to their high content of nutrients, low cost, and versatility, are a popular food item for all cultures. Incorporation of CLA in eggs could lead to alternate sources of CLA for humans. However, chicken eggs are also rich in saturated fatty acids and cholesterol, and the consumption of CLA-rich eggs on human and animal health needs to be investigated.

My current project addressed the following issues: 1) the effect of feeding CLA to hens on the fatty acid profile, cholesterol, and vitamin E content of egg yolks, and 2) the effect of feeding CLA-rich egg yolk powder to hamsters on lipid metabolism, lipid peroxidation, and antioxidant enzyme activities.

I fed CLA oil to hens, which resulted in a significant incorporation of CLA isomers in eggs laid by these hens. One serving (two eggs) of CLA-enriched eggs could provide over 560 mg of dietary CLA. No effect of CLA was observed on egg cholesterol or quality or on hen performance, but storage of CLA-enriched eggs over three weeks reduced the vitamin E content (alpha- and gamma-tocopherol).

I also investigated the effect of CLA-enriched yolk powder on lipid metabolism in hamsters. Eggs were collected from

hens fed diets containing 1% (low CLA) or 2% (high CLA) CLA-enriched diets. Hamsters were fed a diet containing 20% of CLA-enriched yolk powder that provided about 30% of fat energy as recommended by national dietary guidelines. Except for CLA, the composition of the dietary fatty acids and cholesterol resembled that in a typical Western diet.

The high-CLA diet decreased total fat in hamster livers. A significant increase in CLA fatty acid incorporation was observed in the liver and spleen with a concomitant reduction in omega-6 fatty acids, including arachidonic acid, the precursor of proinflammatory eicosanoids. The incorporation of mono-unsaturated fatty acids was lower in the spleen and liver of hamsters fed low- and high-CLA diets compared with regular egg yolk or casein control diets. CLA-rich yolk feeding also resulted in an increase in saturated fatty acids in tissues. Hepatic triglycerides were lower in hamsters fed the high CLA diet.

The changes in lipid content and liver fatty acid profiles were associated with a decrease in lipid oxidation products, such as malondialdehyde, which was lower in the livers of hamsters fed CLA yolk-based diets. No difference was observed in plasma prostaglandin E₂ concentration or F₂-isoprostanes, another marker of lipid oxidation. I then measured changes in the antioxidant defense system by assessing activities of superoxide

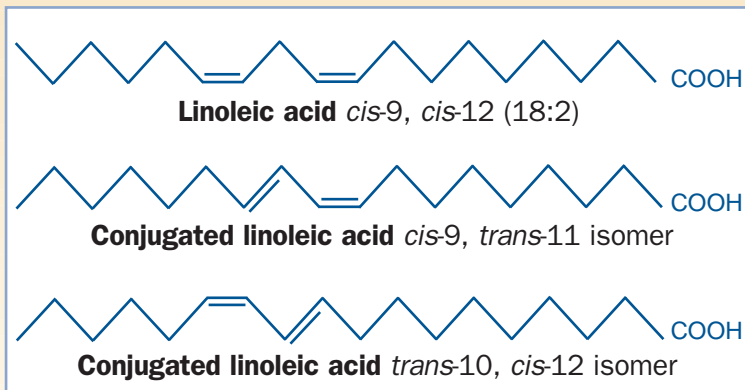
dismutase, glutathione peroxidase, and glutathione reductase. The activities of these endogenous antioxidant enzymes were increased in the livers of hamsters fed CLA-rich egg yolk. Fecal total lipids and total cholesterol were higher in hamsters fed high CLA. Finally, there was no change in aortic plaque formation associated with feeding the hamsters CLA-rich egg yolks.

The results of this study indicate that in hamsters, CLA from a non-ruminant source like eggs can be increased without any adverse effects. We observed a significant increase in antioxidant activity associated with CLA feeding, which may suggest an antioxidant action of CLA. Fecal lipid and cholesterol excretion also suggest that egg yolk CLA modulates lipid and cholesterol metabolism. However, the exact mechanism of dietary CLA in lipid metabolism needs to be further explored.

CLA consumption in Western diets may be increased by CLA-enriched eggs used in foods like mayonnaise, pasta, salad dressings, baked goods, or other poultry meat products. The success of such CLA-modified egg products will depend upon acceptable sensory characteristics and stability during cooking, storage, and processing. These factors have yet to be investigated in detail.

Based on these preliminary results obtained through research supported by an LPI pilot project grant, I plan to submit a grant proposal to the USDA to investigate the functional food attributes of CLA-enriched chicken eggs in humans. These findings will generate new fundamental knowledge about functional food strategies to prevent the progression of chronic diseases like heart disease.

Structure of Linoleic Acid and CLA





Developments

Scott Palmer
LPI Director of Development

One of the things I like most about this job is getting to know some of the great people who support the Institute, including Dorothy Epstein and Sonia Penich. Dorothy and Sonia both lived in New York City not more than a few miles from one another. Both were compassionate, strong-willed, independent women with backbones of steel who overcame numerous challenges to become inspirations to their respective families and friends.

Dorothy was born in the Bronx in 1913, and Sonia was born in Belgrade, Serbia, three years later. Dorothy and her family faced many challenges during the Great Depression, and Sonia survived the Nazi occupation of Belgrade during WWII.

In 1962, Sonia fled her native Serbia to escape the communist repression of Marshall Tito. She lived in Paris for several years before coming to the United States in 1974, where she learned to speak English and went to college. After graduating from Columbia University, she worked for the New York City Criminal Court Library, where she spent the next 30 years as an employee and volunteer.

Dorothy graduated from Hunter College in 1933. At different times in her life she was a social activist, a union organizer, and an entrepreneur. It was during her tenure as the first female president of a health food and vitamin production company that her passionate and long-term support of Linus Pauling and the Institute he founded began. She was one of the Institute's strongest advocates and was always looking for ways to advance the cause of health research.

When Dorothy retired at the age of 76, she became an advocate for the rights of seniors. Nine years later she started writing her memoirs. Sometime next year her book, *A Song of Social Significance*, will be published by Ben Yehuda Press of Teaneck, NJ. It is a remarkable story.

Both women became supporters of the Linus Pauling Institute of Science and Medicine shortly after it was founded by Dr. Pauling in 1973 and, unfortunately, both passed away this past spring within a few weeks of each other.

Each of them found different ways to support the Linus Pauling Institute. Through her will, Sonia provided a substantial bequest to LPI. Dorothy had been making large annual gifts to LPI for some time, including a very generous gift shortly before her death.

Today, the Linus Pauling Institute is a world-renowned research center on micronutrients and phytochemicals, due in large part to the support we have received from people like Dorothy and Sonia. The financial contributions we receive from our many friends provide the Institute with the critical resources to grow and explore new scientific boundaries. Your support *does* make a difference.

Within the past few months, Congress has made it easier for the average person to support their favorite not-for-profit organization by allowing them to make a gift from their individual retirement account (IRA). The Pension Protection Act of 2006 created a significant tax incentive for donors who are 70½ years or older to donate up to \$100,000 per year of their IRAs to organizations like the Linus Pauling Institute at Oregon State University. Under this new law, you can make a gift using funds from your IRA without undesirable tax effects. A charitable gift to the Linus Pauling Institute will count toward satisfying mandatory withdrawal amounts.

Thousands of people created IRAs when the concept was first introduced years ago and have been contributing to them every year. Those annual contributions, coupled with compounded, tax-free annual growth, have resulted in large IRA accounts for many people who have retired. Prior to this recent legislation, it was difficult for someone to donate all or a part of their IRA to a charitable organization.

Now, gifts from IRAs can be made simply and without tax complications. Plus, you can make the gift now—while you are living and able to witness the benefits of your generosity. You may contribute funds this way if:

- You are age 70½ or older.
- The gift is \$100,000 or less each year.
- You make the gift on or before December 31, 2007.
- You transfer funds directly from a traditional IRA or Roth IRA. Donors should *not* take the distribution themselves and subsequently write a check to a charity.
- You transfer the gift outright to one or more public charities like the Linus Pauling Institute at Oregon State University, but not to supporting organizations or donor-advised funds.

Some of our supporters are already taking advantage of this new law to make their annual gifts to the Linus Pauling Institute. If you would like more information about how to provide a gift to the Institute from your IRA, please don't hesitate to contact me.

In the meantime, I will continue doing what I like best—spending time on behalf of the Linus Pauling Institute with remarkable people like Dorothy Epstein and Sonia Penich, who cared deeply about their families, friends, and community.

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LPI

LPI is deeply grateful for the bequests we have received from the following friends

John F. Holterhoff
David Holtzman
Sonia Penich
Karla Pepe
Sylvia Robb

Helen J. Sagar
Charles Saltzman
David L. Soltker
Robert B. Stewart



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CONFERENCE TOPICS

Health promotion and disease prevention by lifestyle and diet, including vitamins, minerals, and phytochemicals; and the role of oxidative stress and antioxidants in human health and disease

Look for these informative articles inside!

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

Micronutrient Research for Optimum Health

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