

OSU Oregon State University

The Linus Pauling Institute

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



From the Director

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

In my last column I told you about the strategic planning process that the Linus Pauling Institute embarked on about a year ago and promised to update you on the emerging key goals and initiatives. We are now in the process of finalizing the plan, which will be released in May. As we move on to the all-important implementation phase, there will be numerous changes in how we do business here in the Institute. For instance, future Research Newsletters will be published online only, to allow for greater flexibility in content, linking to other articles and web-based resources, and to save expenses for printing and mailing, which are considerable for over 14,000 copies. Therefore, if we do not have your email address and you would like to continue to get our Newsletter, please send an email to lpi@oregonstate.edu. If you do not have access to email and would like to continue to receive a hard copy of the Newsletter, please use the enclosed card to let us know. We'll be happy to send you a copy by regular mail.

As part of the strategic plan, we first developed a new vision and mission for the Institute. Our vision is "Discovering how to live longer and feel better," which many of you will recognize from the title of Linus Pauling's best-selling book touting the many health benefits of vitamin C and other dietary supplements as part of a healthy diet and lifestyle. Our vision thus links back to our founder and his ground-breaking concept of orthomolecular medicine—the right molecule at the right concentration to achieve optimal health and prevent disease—and at the same time looks ahead to what we are trying to achieve through our work, as explained in our new mission statement:

The mission of the Linus Pauling Institute is to promote optimal health through cutting-edge research and trusted public outreach. To accomplish this, we will:

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Metabolizing Drugs and Toxins

*An Interview with
Sharon Krueger, Ph.D.
Assistant Professor (Senior Research)*

Q. Where did you earn your doctorate?

A. I earned my doctorate in genetics and crop science from Oregon State University.

Q. Did you work on plant breeding at OSU?

A. I did. I got my master's degree in plant breeding in Wisconsin, and then I moved here to pursue my Ph.D. My master's degree was involved with traditional genetics and plant breeding, and I wanted to come to OSU to study genetics at a population level. I was working with a rather obscure plant called *Cuphea*, which was being developed for its unique spectrum of seed oils.

Q. How did you become involved in the Department of Fisheries and Wildlife?

A. When I was here as a Ph.D. student, I was diagnosed with a rare genetic enzyme deficiency that causes a metabolic muscular dystrophy. I was preparing for a career as a Mendelian geneticist doing traditional crop breeding that would have placed me out in the field, but that was not going to be compatible with my condition. Although my underlying interest was in genetics, I really wanted to change my research focus to work on metabolism, which would be compatible with the constraints of muscular dystrophy. That led me to Fisheries and Wildlife, where I could establish my ability to work with animals.

Q. How did that lead to you working in environmental and molecular toxicology?

A. I was aware of Dr. Williams' work—who's now an LPI Principal Investigator—and he had an opening for someone to work on the metabolism by enzymes of both endogenous and exogenous compounds, including pharmaceutical drugs and pesticides.

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- Discover basic mechanisms underlying the biology of aging and the causes of metabolic and age-related diseases
- Develop effective approaches to slow aging and postpone metabolic and age-related diseases through diet, micronutrients, and phytochemicals
- Advance the principles of healthy living and healthy aging in the public arena, thereby empowering people everywhere to add years of health and vitality to their lives

Advancing healthspan, not just lifespan, is our passion.

The overarching theme emerging from our strategic plan is for LPI to create, communicate, and implement new knowledge that will advance human healthspan. Health is defined here not just as the absence of disease but as optimal health, vitality, and resiliency into advanced old age, free of disability and the deficits of daily living. The focus is on quality of life—healthspan, “feeling better”—and on closing the widening gap between lifespan and healthspan in the US population and around the world.

The strategic plan identifies four major goals for the Institute. The first is to establish LPI’s leadership role in advancing human healthspan through cutting-edge research. This goal will be pursued through our research programs in healthy aging, cancer prevention and intervention, and cardiometabolic disease prevention, supported by cross-cutting facilities offering state-of-the-art tools and technologies. One of these core laboratories will develop unique screening models to identify dietary factors and other novel compounds that advance healthspan.

The second goal is to diversify and increase funding for LPI research and outreach. Like so many other

academic institutions around the country, LPI has experienced a steady decline in federal research funding over the past five years. While we will continue to pursue grants from the National Institutes of Health, it will be imperative for LPI to aggressively diversify and increase funding from other sources. We will have to explore business opportunities, pursue grants from private industry, and increase philanthropic support to sustain our current work and realize the many new initiatives of our strategic plan.

“Discovering how to live longer and feel better”

The third goal is to communicate the LPI message and raise LPI’s visibility. To this end, we will develop and implement a comprehensive communications and marketing plan that will advance knowledge, understanding, and support of LPI, especially among our donors, the general public, health professionals, and the media. We will also realize the full potential of the Micronutrient Information Center (lpi.oregonstate.edu/infocenter)—the Institute’s “public face”—our comprehensive, online database for scientifically accurate information on the roles of micronutrients and other dietary factors in health and disease.

Finally, we will help improve public health through community engagement. We will support and expand the efforts of LPI’s Healthy Youth Program to empower youth and their families to achieve lifelong health and wellness. An important initiative will be to validate and disseminate the high-quality outreach programs developed by the Healthy Youth Program so that they can be duplicated in communities around the country.

You will hear a lot more about all the exciting new initiatives going on here at LPI over the years to come. In the meantime, enjoy reading this Newsletter, and as always, feel free to contact us with any questions you may have about our work. Here’s to your good health and a great spring and summer! [LPI](#)

Q. *When did you become a research assistant professor in LPI?*

A. In 2007.

Q. *You’ve been interested in a group of enzymes, the flavin-containing monooxygenases, or FMOs, for a long time. What do these enzymes do, and how many of them are there?*

A. There are five different forms of flavin-containing monooxygenases with activity in humans. They are

found where you are first exposed to a drug or a foreign chemical—the skin, lungs, liver, kidneys, and intestine. All of these organs contain flavin-containing monooxygenases.

Q. *What do FMOs do in the body?*

A. FMOs are similar to another family of enzymes called the cytochromes P450s. They are located in similar places in the cell and have similar functions—metabolizing foreign substances like drugs and toxins. FMOs and cytochrome P450s add an oxygen atom to these molecules that makes the resulting metabolite more readily excreted from the body.

Q. What stimulated your interest in FMOs?

A. I wanted to work on enzymes that were important in metabolism.

Q. Are there ethnic or gender differences in the activity of FMOs?

A. There are five different families of FMOs in humans, and much of the work that I've done has been with FMO form 2. This form is primarily located in the lungs of humans and most other mammals. There is a major mutation in this particular enzyme in people of African or Hispanic descent. Everyone else who has been tested so far has a version of the FMO2 that is nonfunctional. Other FMOs also have different versions, called polymorphisms, but none are as widespread as the FMO2 variant.

Q. How prevalent is the FMO polymorphism in Hispanics or African-Americans?

A. It can run from about five to seven percent of Hispanics, and among people who are from Africa, it is highly dependent upon the specific region. Active FMO is most prevalent in sub-Saharan Africa, where there can be an incidence of up to 50%.

Q. Can these genetic polymorphisms or differences be easily detected by blood tests or some other assay?

A. The assay that we have used requires the isolation of DNA from blood or saliva. We can then amplify the DNA to detect specific sequences.

Q. What are the consequences of this genetic heterogeneity of FMOs in terms of drug therapy or exposure to toxins?

A. The FMOs primarily metabolize nitrogen- and sulfur-containing compounds, including some antidepressants that are based on tricyclic amine structure and some sulfur-containing antibiotics. Genetic heterogeneity contributes to differences in drug response.

Q. Does that mean that people who have this variant, the polymorphism in FMO2, process drugs or toxins in a more effective or less effective way than people who do not have the mutation?

A. On one hand, oxygenation by FMO may speed up the excretion of the metabolite in the urine. But FMO metabolism is sometimes required to activate a molecule and, therefore, may generate a more toxic metabolite.

Q. Why?

A. When FMOs metabolize sulfur-containing compounds, they generate a sulfenic acid metabolite that can cycle with glutathione—an important antioxidant that protects cells. This leads to glutathione depletion *in vitro* and potentially *in vivo*, as well.

Q. Does the activity of FMOs change with age?

A. We probably can't say that FMO2 changes a lot with age, but there are other FMOs that do change with age.

There is a developmental switch from FMO1 activity in the fetal liver to FMO3 activity in the adult liver. FMO1 declines very quickly—within three days—after birth. FMO3 is detectable by one to two years after birth and approaches adult levels during the teenage years.

Q. Are FMOs affected by diet?

A. Yes, they can be. There have been a couple of studies where people were given about 300 grams (10.6 ounces) a day of Brussels sprouts to eat, and that depressed FMO3 activity in the liver.

Q. Does that have to do with the indole-3-carbinol in the Brussels sprouts?

A. Yes, that is what we think, since indole-3-carbinol reduces FMO activity in animal studies.

Q. Another detoxification system is the cytochrome P450 monooxygenase (CYP) family that metabolizes foreign substances, including antibiotics. Do FMOs act on the same kinds of xenobiotics?

A. They do, and we spent a number of years determining which FMOs could metabolize different compounds. Very often we also have to determine whether CYPs metabolize the same compounds.

Q. And metabolism prepares them for excretion from the body?

A. Right. This is a system your body has to help deal with foreign compounds. The object is to render them less toxic and then to eliminate them. The FMOs metabolize many plant alkaloids and synthetic drugs but don't act on a lot of known endogenous compounds. Cytochrome P450s are a lot more abundant and, as a family, more diverse in the compounds that they metabolize. Some FMOs we don't know much about. For instance, FMO5 does not metabolize drugs, although it can be present in quite high amounts. It is probably a product of the ancient ancestral form of the gene, but its endogenous role is not known.

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Q. *You mentioned that the FMOs act on xenobiotics or toxicants that contain sulfur or nitrogen. Is there anything else special about certain toxicants, environmental pollutants, or drugs that target them from metabolism and excretion from the body by FMOs?*

A. Aside from the targets provided by sulfur-, nitrogen-, and some selenium-containing compounds, the size and bulkiness of the compound is a major constraint on its metabolism by FMOs.

Q. *Is there an example of a toxicant, environmental pollutant, or drug that FMOs target that is not targeted by the cytochrome P450 system?*

A. Ethionamide, which is a drug used to treat tuberculosis. It was not metabolized by cytochrome P450s in our studies. FMO3 is the primary enzyme that metabolizes sulindac sulfide, an NSAID. FMOs also metabolize the pesticide phorate very quickly, with the highest activity we have ever been able to measure.

Q. *What inhaled drugs might be metabolized by FMOs?*

A. FMO2, the form in the lungs, metabolizes some of the compounds in cigarette smoke like nicotine.

Q. *Do FMOs play a role in protecting against colorectal cancer in patients with familial adenoma polyposis (FAP) who take the drug sulindac?*

A. Yes. Sulindac seems to be more effective in FAP patients with FMO3 genetic variants that reduce activity—they had fewer colon polyps. Dr. Gayle Orner, who used to work at the Linus Pauling Institute, and I had a small project to look at whether we could use cruciferous vegetables like Brussels sprouts to alter the level of the FMO3 in the liver to reduce the rate of the sulindac metabolism.

Q. *How does FMO3 metabolize sulindac?*

A. Sulindac is an oxygenated drug: sulindac sulfoxide. When that's swallowed and passes to the intestine, the microbes in the gut reduce it to sulindac sulfide, which is the active form of the drug. This reduced form gets absorbed into the blood stream, circulates back to the liver where the FMO3 is, and then FMO3 re-oxidizes it. It's a recycling process that happens several times before the drug is finally eliminated from the body.

Q. *Does that mean that people could take less sulindac for an effective outcome?*

A. People with FAP develop resistance to sulindac. So there's less of a focus on taking less and more on getting a better outcome from the dose by being able to take it longer more effectively. Theoretically, higher sulindac sulfide levels would be achieved with certain mutations, and this would increase effectiveness.

Q. *With Dave Williams of LPI and others you've investigated the role of FMO's metabolism of tamoxifen, a drug used to treat breast cancer. What did you find?*

A. Tamoxifen is oxidized by FMO, a reaction that detoxifies and eliminates tamoxifen. In contrast, cytochrome P450s hydroxylate and activate tamoxifen. FMO metabolism could reduce non-target toxicity but might also reduce efficacy, while CYP metabolism could increase both drug efficacy and toxicity.

Q. *Also with Dave Williams and others, you've studied the effect of FMOs on the metabolism of the anti-tuberculosis drug ethionamide in mice. You mentioned that FMOs metabolize this drug.*

A. Yes, the FMOs, including FMO1, FMO2, and FMO3, metabolize ethionamide. We were particularly interested in looking at this because people who have active FMO2 are primarily in sub-Saharan Africa. The primary incidence of tuberculosis is also in sub-Saharan Africa, so there's a convergence of TB and FMO2 metabolism.

Q. *What is the practical outcome of that observation? Does this have any impact on drug therapy for TB patients?*

A. Yes, it could very well. Ethionamide is a sulfur-containing drug, and this is one of the cases where instead of seeing detoxification, something more toxic might be formed. Ethionamide is a pro-drug that needs to be activated to have bactericidal activity. FMOs add an oxygen to form sulfenic acid, which converts more slowly to sulfinic acid. But that first step to form sulfenic acid can lead to depletion of glutathione, as I mentioned, by cycling—forming the oxidized version—which leads to an almost suicidal cycle of continuous depletion, at least *in vitro*. In Africans who have the active form of the enzyme FMO2, oxidative lung damage might result from taking the drug and might reduce the amount of ethionamide available to combat the TB mycobacteria.

Q. *Would that suggest that suppression of FMO expression might be beneficial to people undergoing drug therapy for TB?*

A. It may reduce the amount of oxidative stress to the lung.

Q. *Are there any dietary interventions to suppress FMO activity?*

A. I'm not aware of any, other than cruciferous vegetables, that contain indole-3-carbinol and suppress FMO3. I don't think they have the same effect on FMO2. If you could reduce the level of FMO3 in the liver, then you might be able to deliver a higher therapeutic dose of the drug to kill the mycobacteria, as FMO3 also metabolizes this drug. Mycobacteria are actually quite interesting themselves because they also have an FMO—unlike most microorganisms—and FMO metabolism of the ethionamide by mycobacteria is responsible for their death.

Q. What important questions about FMOs remain to be answered?

A. We need to get more definitive information on the benefits of regulating FMO3 in familial adenoma polyposis and in cancer. There are still a lot of questions remaining on the effect of FMO2 in tuberculosis. We've had some promising results, although nothing yet as clear-cut as we would like to see. A third of the world's population is infected, so there are a lot of people who are on drug therapy at any given time. There wouldn't be as much application in this country because the number of people that have the active form of FMO2 is not high.

Q. You've worked on the dietary protection against cancer in the offspring of pregnant mice exposed to environmental carcinogens. What dietary compounds might be useful in that role?

A. The long-time interest in the Williams lab is in crucifers, such as broccoli and Brussels sprouts, that contain indole-3-carbinol. Our recent studies with LPI's Emily Ho, Rod Dashwood, and Dave Williams have included sulforaphane, which is typically found at higher levels in broccoli, whereas indole-3-carbinol is at higher levels in Brussels sprouts.

Q. In those experiments, did you feed the pregnant mice the purified compound or the vegetable?

A. Our last study compared the effects of the purified compounds to a vegetable extract. In our particular model, the highest benefit came from the indole-3-carbinol alone.

Q. What carcinogen were these mice exposed to?

A. They were given dibenzo[*def,p*]chrysene, a polycyclic aromatic hydrocarbon (PAH) formed from combustion in coal-burning plants and in vehicles. PAHs are common air pollutants and known human carcinogens.

Q. Could they be responsible for causing cancer in children whose mothers may have been exposed to them when pregnant?

A. It's possible that they are linked.

Q. How easily do you think that the results from these rodent studies can be extrapolated to people?

A. Dr. Williams developed a very sensitive detection method so that you can give a carcinogen to a human at a level so small it's less than what you would get in your daily exposure in the environment. The carcinogen can be tagged with a radioactive isotope with less radioactivity than what you would get flying across the country or eating five bananas. We collect blood and urine for three days after the carcinogen or similar compound has been swallowed. Extracts of those samples are sent to Lawrence Livermore National Laboratory, where they have an accelerator mass spectrometer that very sensitively measures the radioactive signature. This gives our group the ability to start looking at the kinetics of metabolism in a human being at a realistic exposure level. So for the first time, we're not doing a

rodent study where you give an astronomical dose of the carcinogen and then extrapolate down in a linear fashion to commonly encountered environmental levels.

Q. Are you planning a dietary intervention to see if indole-3-carbinol or cruciferous vegetables interfere with the metabolism of these carcinogens and offer some protection?

A. We are in the process now of working on the investigational new drug protocol to submit to the FDA. Once we get approval from the FDA and OSU's Institutional Review Board, we will probably get started in about two or three months. We will have a small group of human volunteers who will swallow a small dose of radioactively labeled phenanthrene, which is a non-carcinogenic polycyclic aromatic hydrocarbon. We'll collect blood and urine for three days to learn the fate of the chemical. Then there will be a dietary wash-out period. We will ask the subjects not to consume any cruciferous vegetables during the study, aside from what we provide. After the first round we will determine the metabolic fate of phenanthrene when the subjects are not consuming crucifers, then they will take phenanthrene following consumption of indole-3-carbinol.

Q. What do you expect to find?

A. We would expect to find that phenanthrene will be differently metabolized when indole-3-carbinol is present. Indole-3-carbinol should alter the amounts of different phenanthrene metabolites produced and possibly their time course, as well.

Q. If phenanthrene is around for less time, then the probability for DNA damage is decreased?

A. Correct. In the third cycle of the study, after another wash-out period, the subjects will go through the same protocol again but will eat Brussels sprouts every day for one week before they take the phenanthrene. So we will be able to compare the effects of the purified compound with the compound in its food matrix.

Q. Most recently you developed an interest in high-dose intravenous vitamin C to treat cancer. Is it similar to the work that Linus Pauling and Ewan Cameron did with terminal cancer patients back in the 1970s?

A. It certainly uses that as the foundation. It involves a subject of interest to LPI Principal Investigator Joe

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Newly Updated Articles in the Micronutrient Information Center

Articles on vitamin C, riboflavin, vitamin B₁₂, fluoride, and copper have been updated on the Micronutrient Information Center (lpi.oregonstate.edu/infocenter). Pending updates include folate, vitamin D, magnesium, and essential fatty acids. We encourage donations to help maintain and expand our MIC!

Beckman, who has a series of copper compounds. Copper ATSM is a compound that chelates or binds copper in either its oxidized (Cu II) or reduced (Cu I) form. Since it accumulates in tumors, a radioactive version is used for tumor imaging. Joe Beckman is using it in his research with an ALS animal model, and he has produced a whole series of other copper-chelating analogs. Joe, Balz Frei, and I want to see if these compounds might have anticancer effects—inhibit tumor progression—in rodents. Copper is known to concentrate in tumors. It's believed that is due to the acidic environment in a tumor and its hypoxia or lack of oxygen. The hypoxia drives the tumor to become more acidic, and that may explain why it retains copper. The most aggressive tumors have higher levels of copper than less aggressive tumors. The copper ATSM increases copper levels in tumors. In the cell, oxidized copper can be reduced and, once reduced, can dissociate from ATSM and become trapped. Copper might then interfere with mitochondrial function or generate hydrogen peroxide. If vitamin C concentrations are high, an additional mechanism could be copper-dependent oxidation of vitamin C, resulting in the formation of hydrogen peroxide that then generates radicals that will kill cancer cells.

Q. Mark Levine's research at the National Institutes of Health showed that high extracellular concentrations of vitamin C generate hydrogen peroxide that then goes into the cancer cell and causes cell death. Are you proposing that the hydrogen peroxide could react with copper inside cells to make radicals that kill cancer cells?

A. I think that copper-dependent oxidation of vitamin C to generate hydrogen peroxide could happen both inside and outside the cell. The first step for us is to show that these new compounds are not toxic in rodents. Then we want to find out if they affect tumor growth rate. Lastly, we want to understand the mechanism and extend the work to humans if it's promising in rodents. We will first do some cell culture studies, then we'll take the promising compounds and put them into immunocompromised mice that can be a host for a human tumor, called a xenograft. These mouse xenografts have turned up a lot of compounds that decrease the tumor growth rates or tumor size, but many aren't successful in subsequent human trials. On the other hand, there are some reports of very good outcomes when part of a tumor from a cancer patient was implanted into mice. Then the mice are tested on different therapies to look at which therapy knocks back that particular tumor. That mouse-guided treatment has led to some good treatment results.

Q. What other research projects are you planning?

A. The copper literature has really been a fascinating story, as well as investigating xenografts and how they are

used. I've been very intrigued by various approaches to chemotherapy, where most people get very high doses and experience toxic effects and damage to normal organs. There is some interesting literature suggesting that you can have very good outcomes if you do long-term management of cancer at sustained low levels of chemotherapeutic drugs rather than shorter bursts of very high levels. I am interested in looking at how these drugs, including copper, might be combined with vitamin C for a good outcome with fewer toxic side effects. Whether vitamin C is compatible with some traditional chemotherapy is still debated and is an area that warrants additional study.

Q. Pauling and Cameron didn't face that obstacle with their patient population in the 1970s because the cancer patients getting high-dose vitamin C were people who had been ruled out for chemotherapy, surgery, or radiation, so there wasn't any problem with drug compatibility.

A. In cancer, we really want to prevent the disease. If cancer occurs, we want to delay it and slow progression. I have an interest in what we do when someone already has a tumor because cancer is, unfortunately, still a frequent diagnosis. It would be fantastic if we could use intravenous vitamin C as a sole therapy, but I think it could be very important and synergistic in combination therapy.

Q. How has your work been funded?

A. The FMO work has been funded by the National Institutes of Health through the National Heart, Lung and Blood Institute, and the National Cancer Institute funded the work at LPI comparing dietary interventions and supplements. The work on PAH carcinogens has been funded by the National Institute of Environmental Health Sciences.

Q. How do you enjoy your free time outside the lab?

A. I like to go birding and enjoy visiting gardens. Oregon is a wonderful place for those activities, with mountain meadows, the Iris Gardens, and ocean beaches. At this time of year, I'm often birding over in the Albany-Millersburg Talking Water Gardens. I moved from Wisconsin, in part, because of the cold winters with snow and ice and the hot, humid summers with lots of mosquitos.

Q. What is it about LPI that you enjoy?

A. As soon as I heard that LPI was moving to OSU, I wanted to be a part of it. I come from a family that embraced the idea of good nutrition and supplement use. I was raised on a farm in Wisconsin where we grew most of the food we ate. Having a rare metabolic muscular dystrophy made me want to make the most of my health. The research that we do in LPI is about how we can improve everybody's health, and there is a real satisfaction working with a group of researchers whose shared goal is improved health. **LPI**

The Case is Far from Closed for Vitamin and Mineral Supplements!

In their recent editorial in the journal *Annals of Internal Medicine*, “Enough Is Enough: Stop Wasting Money on Vitamin and Mineral Supplements,” the authors (Guallar, Stranges, Mulrow, Appel, and Miller III) conclude that “we believe that the case is closed—supplementing the diet of well-nourished adults with (most) mineral or vitamin supplements has no clear benefit and might even be harmful.” It appears that the authors themselves weren’t quite convinced of their conclusions because they added numerous qualifiers, such as “we believe” and “well-nourished adults,” and put “most” in parentheses.

While a well-balanced diet is the best way to get almost all of one’s essential nutrients, the reality is that Americans don’t get enough of them every day through diet alone. From the National Health and Nutrition Examination Survey (NHANES), we know that the large majority of the US population is not “well-nourished” and falls short of getting all of their vitamins and minerals from their diet in levels recommended by the Institute of Medicine’s Food and Nutrition Board. For example, more than 93% of US adults 19 years and older do not meet dietary intake recommendations (called Estimated Average Requirement, or EAR) of vitamins D and E, 61% for magnesium, about 50% for vitamin A and calcium, and 43% for vitamin C. Certain subpopulations, including older adults, African Americans, and the obese, have increased needs for some micronutrients. Other studies have shown that people who take a daily multivitamin/mineral (MVM) supplement with the recommended doses of most vitamins and minerals can fill most of these nutritional gaps safely and at very low cost—a year’s supply of a high-quality MVM can be purchased for less than a nickel a day.

In contrast, only a very small, non-significant fraction (0.1%) of US adults exceeds the Tolerable Upper Intake Level (UL) from diet and supplements combined for vitamin E, putting Guallar et al.’s claim that “vitamin E ... supplements increase mortality” in perspective. Contrary to the impression that the authors give in their editorial, the US population is inadequate in many vitamins and minerals, a result of the calorie-rich and nutrient-poor dietary pattern of Western populations, rather than over-consuming MVM and other dietary supplements.



Vitamins and nutritionally essential minerals maintain normal cell function, metabolism, growth, and development through their roles as essential cofactors in thousands of enzymatic reactions and other biological processes—their main biological function is not to prevent or treat chronic disease. Nevertheless, the largest and longest randomized controlled trial (RCT) of MVM supplements conducted to date, the Physicians’ Health Study II (PHS II), found a significant 8% reduction in total and epithelial cell cancer incidence in male physicians and a 12% reduction in total cancer incidence, excluding prostate cancer. The PHS II also found a significant 9% reduction in the incidence of total cataract. These findings of PHS II are consistent with those of several other RCTs and are even more impressive given the fact that the conventional RCT design is strongly biased against showing benefits of essential nutrients, in contrast to pharmaceutical drugs. Unlike drug trials in which the control subjects have none of the drug in their bodies, subjects in vitamin trials will always have enough of the micronutrient present to prevent severe deficiency diseases—hence, there is no true placebo control group in such studies.

Furthermore, most published vitamin studies and trials have not measured the concentration of the vitamin in the subjects being tested but only assessed intake. Many factors, including age, gender, disease, ethnicity, and genetic differences or polymorphisms, affect the concentrations of ingested micronutrients in the blood, organs, and tissues of humans. Without those measurements, it’s difficult to estimate the effect of supplemental micronutrients.

Taking a daily MVM supplement will not only help fill the known nutritional gaps in the average American diet, thereby assuring normal biological function and metabolism and supporting good health, but may also have the added benefit of reducing cancer and cataract risk—which no existing pharmaceutical drug can do. The benefit-to-risk ratio also favors MVM supplementation, with plausible or demonstrated health benefits and little risk. To call “the case ... closed” and label MVM supplements as useless, harmful, or wasteful is unscientific and does not serve public health. [LPI](#)



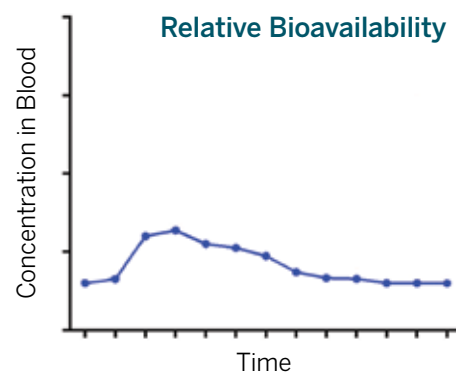
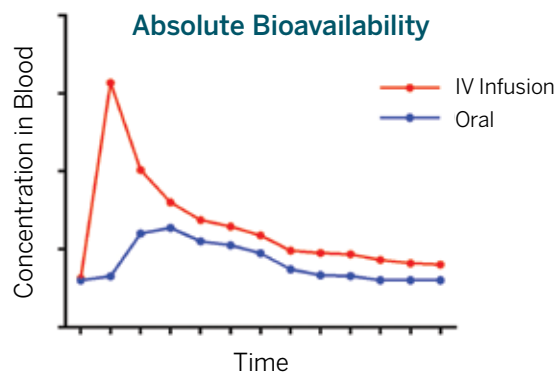
What is Bioavailability?

Alexander Michels, Ph.D.
LPI Research Associate

Bioavailability is an important factor in understanding nutrition and micronutrient research. However, the concept of bioavailability and its broader implications may be unclear or confusing to many. In a broad sense, bioavailability is a measure of the amount of a substance that is absorbed into the blood stream after it enters the body. When referring to nutrients, micronutrients, and other dietary factors, bioavailability usually refers to absorption after oral administration, such as eating a food or taking a supplement. Depending on the question being addressed, researchers may use different methods to assess bioavailability and to answer questions about the behavior of a compound after it enters the body.

Many factors can affect bioavailability, including the current status of the substance in the individual, general health of the subject, delivery mechanisms, and interactions with other substances that may increase or decrease absorption. For example, vitamin C enhances the absorption of dietary nonheme iron (increasing the bioavailability of iron), but the absorption of vitamin C is inhibited by the flavonoid quercetin, found in apples, kale, and onions (decreasing the bioavailability of vitamin C). Bioavailability is also a fundamental issue in studies on flavonoids. Since there are no specific transport mechanisms for flavonoids in the gut, the absorption of many of these molecules is slow and unfacilitated, resulting in very low concentrations in the blood stream even after eating foods rich in flavonoid compounds. In many cases, the concentrations of flavonoids achieved are not high enough to have a meaningful biological effect. Therefore, flavonoids are often categorized as being poorly bioavailable. The situation is further complicated by the rapid chemical modification of flavonoids after absorption.

Another example of bioavailability comes from studies on vitamin C by Dr. Mark Levine's research group at the



Accurate measures of bioavailability require measuring blood concentrations of the molecule of interest. *Absolute bioavailability* is the most precise measure of bioavailability. In order to determine absolute bioavailability, separate trials of both oral and intravenous administration of a particular dose of a substance are required. A comparison of the plasma concentrations achieved after oral versus intravenous dosing determines the fraction of the substance that is absorbed through the gastrointestinal tract in relation to the concentration after direct delivery into the blood stream. This fraction is defined as the bioavailability of the substance. These experiments, called pharmacokinetic studies, are very accurate but elaborate and expensive.

Because of these limitations, *relative bioavailability* is often used in research. The experimental trials needed to establish relative bioavailability only require blood sampling over time after delivery (typically oral administration) of the compound being studied, so they are simpler and less expensive. Measuring relative bioavailability is usually sufficient to answer the questions most often asked in research on the human diet.

National Institutes of Health. They performed very careful pharmacokinetic studies using oral and intravenous administration to determine the amount of vitamin C that can be absorbed from supplements provided at different doses to healthy volunteers. They found that all of the vitamin C is absorbed into the blood stream at oral doses up to 200 mg—exhibiting complete (100%) bioavailability. However, at larger doses the absorption declined, suggesting that the bioavailability declined as the dose increased above 200 mg. This phenomenon can be explained by the existence of vitamin C transport molecules on the surface of cells that line the gut. These transporters limit the amount of vitamin C absorbed into the blood stream. Subtle genetic differences, called polymorphisms, between people affect the amount and activity of these transporters, further complicating generalizations about bioavailability.

Overall, the results of studies on bioavailability are vital to our understanding of the variety of molecular mechanisms involved in the absorption of vitamins, minerals, and other dietary constituents. These analyses are critically important to understand the role that micronutrients and phytochemicals play in human health. **LPI**



Briefly . . . Vitamin B₁₂ and Stomach Acid-suppressing Drugs

Victoria J. Drake, Ph.D.
Manager, Micronutrient Information Center

In humans, vitamin B₁₂ or cobalamin is required for only two biochemical reactions in the body: to make the amino acid methionine from homocysteine and to make a compound that is involved in the production of energy and in the synthesis of hemoglobin, the oxygen-carrying pigment in red blood cells. In these roles as an enzyme cofactor, vitamin B₁₂ is involved in DNA synthesis, red blood cell formation, and maintenance of neuron integrity. Inadequate intake of vitamin B₁₂ (dietary sources include food of animal origin and fortified food like cereal) or vitamin B₁₂ malabsorption can lead to deficiency of the vitamin, which impairs DNA and hemoglobin synthesis and damages the myelin sheath covering nerves. Clinical symptoms of vitamin B₁₂ deficiency include megaloblastic anemia and neurologic problems, such as painful numbness and tingling of the hands and feet, difficulty walking, memory loss, and dementia. The anemia can be reversed with vitamin B₁₂ supplementation, but neurologic symptoms may not, especially if they have been present for a long time. Linus Pauling's mother, Belle, died from pernicious anemia in 1926, the same year that Minot and Murphy discovered that liver—a rich source of B₁₂—cured the disease. Belle Pauling suffered from physical symptoms and became irrational and delusional in the late stage, exhibiting “megaloblastic madness.”

Dietary vitamin B₁₂ is bound to proteins and must be released in the stomach for absorption in the small intestine; the acidic environment of the stomach and the enzyme pepsin are necessary to dissociate the vitamin from proteins. Consequently, long-term use of drugs that reduce the production of stomach acid may lead to vitamin B₁₂ deficiency. Pharmaceutical inhibitors of stomach acid production include proton-pump inhibitors (PPIs; e.g., Nexium, Prilosec, Prevacid) and histamine 2 receptor antagonists (H₂ blockers, e.g., Pepcid, Tagamet, Zantac). These drugs are used to prevent or treat gastrointestinal disorders, such as peptic ulcers, Barrett's esophagus, and gastroesophageal reflux disease (GERD), and to treat the rare disorder, Zollinger-Ellison syndrome. Excess vitamin B₁₂ is stored in the liver, and symptoms of deficiency usually manifest only after years of inadequate intake or use of acid-suppressing drugs.

Some, but not all, studies have associated long-term use of PPIs with vitamin B₁₂ deficiency in the elderly—a

population with a high incidence of atrophic gastritis that leads to food-bound vitamin B₁₂ malabsorption because of insufficient acidity in the stomach. A recent case-control study—published in the *Journal of the American Medical Association*—evaluated whether the use of prescription acid-suppressing drugs was associated with the diagnosis of vitamin B₁₂ deficiency in adults of all ages (25,956 cases of vitamin B₁₂ deficiency, 184,199 controls). This study found use of PPIs for two or more years was associated with a 65% higher risk of diagnostic vitamin B₁₂ deficiency compared to nonusers, and this association was much stronger (an eight-fold increased risk) in individuals



Belle Pauling, circa 1915

younger than 30 years compared to older age groups and in women compared to men. The study also found that use of H₂ blockers for two or more years was associated with a 25% increased risk for diagnostic vitamin B₁₂ deficiency compared to nonusers. Although this study examined prescription medications, acid-suppressing drugs are also available over-the-counter at lower dosages. Apparently, no research on the effect of acid-neutralizing compounds like antacids on B₁₂ absorption has been published.

This study was an observational study and therefore cannot establish a cause-and-effect relationship between use of acid-suppressing drugs and vitamin B₁₂ deficiency, but there is biologic plausibility for such an association.

As mentioned above, stomach acid is needed to liberate vitamin B₁₂ from proteins in food; in contrast, stomach acid is not required for absorption of the crystalline form. In other words, individuals with food-bound vitamin B₁₂ malabsorption can readily absorb the crystalline form found in fortified foods and dietary supplements. It seems prudent for physicians to monitor vitamin B₁₂ status in individuals taking acid-suppressing medications long term. The Recommended Dietary Allowance (RDA) for adults is 2.4 mcg/day of vitamin B₁₂; for adults over 50 years old, this amount should be in the form of fortified food or supplements. Due to the higher incidence of food-bound vitamin B₁₂ malabsorption in older adults, the Linus Pauling Institute recommends that adults over 50 years old take 100 to 400 mcg of supplemental vitamin B₁₂ daily. [LPI](#)



Cruciferous Vegetables and Cancer Prevention

*Lauren Atwell, M.S., R.D.
LPI Graduate Fellow*

Cancer is a devastating disease that affects millions of people worldwide, and over 1.6 million Americans are projected to be diagnosed with some form of cancer in 2014. Fortunately, approximately one-third of cancers can be prevented, in part, by lifestyle modifications, including diet. While a healthy diet is made up of a variety of food, consumption of certain food has been linked to specific health benefits, including cancer prevention. In particular, the consumption of cruciferous vegetables (“crucifers,” e.g., broccoli, Brussels sprouts, cauliflower, kale) has been associated with a lower risk for several types of cancer. Among these are breast and prostate cancers, which affect more Americans each year than any other type of cancer. The phytochemical sulforaphane (SFN) has been isolated from crucifers and is thought to be responsible for their cancer-preventing effects. However, it remains uncertain how much SFN should be included in the diet to help protect against cancer. Researchers are currently trying to figure out what levels of SFN are associated with specific health benefits and how best to obtain these levels in the body from the diet.

Several researchers have demonstrated SFN’s cancer-preventive effects in cultured cells and experimental animal models. Yet, verifying these effects in human studies introduces unique challenges that researchers are currently trying to overcome. One of the major challenges in studying the effects of any dietary compound relates to the ability of that compound to be absorbed into the blood stream from the gut and then delivered to sites of action in a bioactive form. This overall process is often termed “bioavailability” (see article on page 8). Absolute bioavailability studies compare concentrations in the blood of a compound consumed orally to those after the compound is injected directly into the blood stream, as the latter bypasses the absorptive processes in the gastrointestinal tract. However, injecting a compound into the blood stream of human subjects is not always safe and is rarely performed. Instead, researchers evaluate the relative absorption of a given compound by measuring levels that appear in the blood following oral ingestion of a food, extract, or supplement containing the compound. Comparisons between food sources and their forms (fresh vs. cooked vegetables vs. vegetable extract) contribute to our understanding of a compound’s relative bioavailability and help to identify potent dietary sources for use in studies evaluating the compound’s health benefits. The bioavailability of SFN is not fully understood, and many researchers have demonstrated that SFN is not equally bioavailable from

various dietary sources or in different forms. Thus, there is a need to improve understanding of SFN bioavailability from the diet to accelerate the study of SFN’s efficacy in disease prevention and treatment.

SFN is not absorbed identically from all dietary sources. Crucifers contain glucoraphanin (GFN), which is the glucosinolate precursor of SFN. Chewing or chopping those vegetables releases an enzyme, myrosinase, that converts GFN to SFN, which is the form associated with cancer prevention. Inadequate activity of this enzyme or the inability of the enzyme to make contact with GFN has been shown to greatly decrease the amount of SFN available from vegetables. Myrosinase is inactivated by heat, so cooking crucifers at high temperatures or for long periods of time diminishes its activity and results in lower SFN absorption. Within crucifers, myrosinase is located in a cellular compartment separate from GFN, so disrupting the tissue matrix by chewing or chopping—thereby exposing GFN to myrosinase—is essential for SFN formation. Different cooking techniques and degrees of chewing affect the amount of SFN absorbed from dietary sources.

Another major determinant of SFN levels is the amount of GFN in the crucifer. GFN levels in vegetables are highly variable due to genetic variations among cultivars, growing and storing conditions, and other factors. Two batches of the same cultivar grown in similar conditions can also have extreme variations in GFN content. This variation and unpredictability are problems when trying to provide the same amount of SFN to participants in a human feeding study or clinical trial. Use of crucifers in these studies would require testing each batch and providing different quantities to each subject, which is not often logistically feasible. For this reason, clinical researchers often use dietary supplements as a source of SFN. However, SFN bioavailability from supplements currently on the market is much lower than from whole vegetables. These supplements contain GFN instead of SFN and are devoid of myrosinase activity. Our lab previously demonstrated about a seven-fold lower SFN absorption from these supplements compared to fresh broccoli sprouts. An important aspect of my work has been to evaluate SFN absorption from a dietary supplement that instead contains preformed SFN, thus bypassing the need for myrosinase activity.

We conducted a human feeding study to evaluate SFN absorption from fresh broccoli sprouts and broccoli sprout extracts (BSE) containing SFN. The SFN-rich BSE was produced by researchers at Johns Hopkins University by treating GFN-rich sprout extracts with myrosinase to convert GFN to SFN. SFN content of the resulting SFN-rich powder was measured, and the powder was packed into gel capsules. Thus, the SFN contained within this BSE should be well absorbed, and BSE supplements should provide more SFN than the GFN supplements we studied previously. Our study enrolled healthy adults, ages 18-50 years, who consumed equivalent amounts of SFN from either fresh broccoli sprouts or BSE supplements. Despite the fact that myrosinase activity within the supplements was no longer an inhibitory factor for SFN absorption,

approximately three-fold less SFN was absorbed from BSE supplements compared to the sprouts. This observation suggests that myrosinase activity is not the only determinant of SFN absorption.

Although derived from fresh broccoli sprouts, the BSE used in our study does not contain all of the plant matter, nutrients, and phytochemicals that are naturally found in intact broccoli sprouts. The BSE was obtained by boiling sprouts, so only water-soluble compounds were retained in the supplement preparation. Also, high cooking temperature may have altered some chemical characteristics. Little is known about how the presence of other dietary compounds in the gut can influence SFN absorption, so it is possible that some of the compounds discarded along with the plant material could enhance SFN absorption. The exact reason for the differences in SFN absorption between broccoli sprouts and the BSE remains unclear. Future studies investigating interactions between SFN and other dietary constituents will be an important area of research.

Although the BSE did not deliver as much SFN as the intact sprouts, they were an improvement over previously tested GFN supplements that lacked myrosinase activity. Despite the large difference in the amount of SFN absorbed between the BSE and sprouts, we did not observe substantial differences in the effects of SFN on certain enzymes and proteins involved in cancer. Thus, these SFN-rich BSE supplements may be acceptable for use in clinical trials. Once they become commercially available to consumers, they may also be an important SFN source for individuals who do not consume cruciferous vegetables. However, the safety and efficacy of this BSE have not been tested in all groups of individuals, and it may not be appropriate for certain populations, such as children, adolescents, elderly individuals, or pregnant or lactating women. Additional work is needed to evaluate the effects of this BSE in these populations and others in order to identify individuals who may derive health benefits associated with their consumption. **LPI**



Did you know . . .

LPI's Dr. Maret Traber Honored



Dr. Maret Traber, a principal investigator in LPI and an internationally renowned expert on vitamin E, received the DSM Nutritional Science Award 2013 in September in Grenada, Spain. Dr. Traber was honored for her "lifetime commitment and scientific achievements in the field of vitamin E research." The DSM judging committee stated, "Professor Traber has been providing seminal work and is a major contributor to our understanding of the role of vitamin E as an essential micronutrient in human metabolism in health and disease."

Dr. Traber, the Helen P. Rumbel Professor for Micronutrient Research, has been with LPI since 1998 following stints at New York University School of Medicine and the University of California-Berkeley. From 1998 to 2000, she served on the Food and Nutrition Board's Panel on Antioxidants and Related Nutrients, which is part of the Institute of Medicine of the National Academy of Sciences, and helped to establish the Dietary Reference Intakes for vitamin E.

Dr. Traber is highly regarded for her work on the alpha-tocopherol transfer protein that specifically recognizes that form of the eight-member vitamin E family for distribution to tissues. She also published landmark studies on the bioavailability of vitamin E and showed, for the first time *in vivo*, that vitamin C recycles vitamin E from its oxidized to reduced form, thereby preserving its antioxidant functions.



The Micronutrient Information Center Now in Spanish

Thanks to a generous donation to LPI from Bayer Consumer Care AG, Dr. Andrew Quest of the University of Chile spearheaded the translation of the articles on vitamins and minerals in the Micronutrient Information Center into Spanish. The Spanish version, available at lpi.oregonstate.edu/es/centroinfo, debuted in February 2013.

We are very pleased that this useful information on the role of vitamins and minerals in health and disease is now accessible to Spanish speakers around the world.



Probiotics and Prebiotics: The Bacteria that Live in Your Gut and Affect Health

Giana Angelo, Ph.D., LPI Research Associate

Over the past 30 years, evidence has accumulated that the microscopic organisms that live in our digestive tract play a prominent role in health and well-being. Although it is a complex relationship with much still to be learned, we know that it is a two-way street: what we eat influences the composition and activity of our gut microbiota, and conversely, the nutritional value of food can be influenced by the microbes that inhabit our gut.

The Gut Microbiota

The gut microbiota refers to the collection of microbial species that live in the lower gastrointestinal tract (see **Terminology** box at right). We are born germ free but quickly colonized by bacteria from our mothers and the environment. Mode of delivery, early microbial exposure, diet, and host genetics have all been implicated in shaping one's microbiota. Our gut microbiota stabilizes by about age three, with trillions of different bacteria inhabiting our gut.

Composition

The composition of the gut microbiota changes rapidly in response to diet. For example, one experiment showed that within two days of eating an animal-based versus a plant-based experimental diet, microbial composition was altered such that certain clusters of bacteria characterized each dietary pattern. Upon withdrawal of the experimental diet, the microbial composition reverted back to its baseline state just as quickly. In addition to diet, incorporating probiotics and prebiotics (see below) are other strategies to modify the composition of the gut microbiota.

Function

The bacteria in our gut provide many important functions for the host. They protect against pathogens, extract nutrients and energy from food, contribute to normal immune function, and synthesize some vitamins, including vitamin K, which is bioavailable to the host. A growing body of evidence indicates that the functional potential of the microbiota may go even farther than these established roles.

Energy balance/obesity. Although we each have a unique bacterial “fingerprint,” these many different bacterial species fall into two major groups: *Bacteroidetes* and *Firmicutes*. The ratio of *Bacteroidetes* to *Firmicutes* inhabiting our colon has been linked to obesity—obese individuals have fewer *Bacteroidetes* and more *Firmicutes* compared to lean individuals. But this ratio can be modulated: after one year on a calorie-restricted diet (either fat- or carbohydrate-restricted), progressive

weight loss in obese subjects was accompanied by a shift in the skewed proportions of *Bacteroidetes* to *Firmicutes* to more closely resemble that of lean individuals.

So how might the proportions of *Bacteroidetes* to *Firmicutes* affect energy balance? By isolating and transplanting the fecal microbiota (which accurately recapitulates the gut microbiota) from obese and lean individuals into germ-free mice, scientists can now study how these microbial signatures influence various aspects of physiology. Recipient mice acquire the body composition of the human donor (i.e., increased body mass and adiposity when transplanted with the obese microbiota). Although the gut microbiota from obese and lean donors differ metabolically, we do not yet know how these

differences may influence energy balance. Certain types of bacteria may be more efficient at generating energy from food or may produce signals that influence satiety and hunger.

Atherosclerosis. Inside the dark, anaerobic environment of the large intestine, bacterial fermentation of undigested foodstuff yields short-chain fatty acids, alcohol, gases, and other small molecules. These metabolic products represent one mechanism by which gut microbes can influence host health.

Bacterial fermentation of dietary choline (found in eggs, turkey, and beef) produces a compound known as trimethylamine (TMA). In the liver, TMA is enzymatically converted to trimethylamine-N-oxide (TMAO). TMAO has been implicated as a potential causative factor in the

TERMINOLOGY

Microbe - a microscopic organism, such as a bacterium, virus, or fungus

Biota - from Greek, meaning “all life”

Microbiota - the collection of microbial species that live on and within an individual

Gut microbiota - the collection of microbial species that live specifically in the lower gastrointestinal tract (colon)

Microflora, gut flora - often used interchangeably with gut microbiota

Microbiome - all of the genes that comprise one's microbiota

Probiotic - live cultures of beneficial microbes that can be ingested as a dietary supplement or food additive

Prebiotic - non-digestible food ingredients that promote the growth of beneficial gut microbes

Antibiotic - a medication that kills microbes

development of atherosclerosis because it increases macrophage cholesterol accumulation and foam cell formation, early events in the development of atherosclerotic plaque. New research suggests that one's bacterial community could affect the amount of TMAO that is produced upon exposure to dietary choline, thereby influencing the impact of diet on cardiovascular disease (CVD) risk. It's too soon to tell, but this "diet-microbiota interaction" could represent a new risk factor for CVD.

Probiotics

Probiotics are live, beneficial microorganisms that can be ingested as a dietary supplement or in food. Yogurt products that state "live and active cultures" on the label contain probiotics. Other sources include naturally fermented foods like unpasteurized sauerkraut and kimchi, and traditionally cultured dairy products like kefir and *acidophilus* milk. Commercial probiotics typically provide *Lactobacilli* or *Bifidobacteria*. Keep in mind that these bacteria colonize the gut only temporarily, making regular consumption necessary to sustain their population in the gut.

While many health benefits are touted (see box), strong scientific evidence currently exists for the use of probiotics for only two indications: as a supplement to antibiotic therapy to prevent acute diarrhea and adverse effects in the intestinal environment and to prevent atopic dermatitis (eczema) in infants.

Prebiotics

Prebiotics are food for gut microbes. A prebiotic cannot be broken down by human digestive enzymes but can be fermented by gut bacteria. These non-digestible substrates are thought to selectively stimulate the growth of beneficial bacteria in the colon.

Food products with prebiotic effects are typically non-digestible carbohydrates. The two compounds most extensively tested and with confirmed prebiotic effects are inulin-type fructans (ITFs) and galacto-oligosaccharides (GOS). Both ITF and GOS selectively increase *Bifidobacteria* and *Lactobacilli*, bacterial strains that are also available as probiotics. ITFs occur naturally in several foods, such as leeks, asparagus, artichokes, garlic, onions, chicory, wheat, bananas, and soybeans. Other sources of prebiotics include honey, oatmeal, red wine, and legumes.

As with probiotics, the influence of a prebiotic ingredient on the gut microbiota is transient. Changes in microbial composition respond rapidly—within 24 hours of exposure—and disappear equally fast upon withdrawal of the prebiotic compound.

Conclusion

We are at any early stage in the discovery process of the effects of probiotics and prebiotics. It is difficult to identify the explicit effects of your microbiota on your health, and no specific recommendations can yet be made.

We do know, however, that there is an interaction—we coexist with these microscopic organisms, and they can help us or harm us depending on how they are treated.

As we await more scientific information, the good news is that following the existing dietary guidelines will provide prebiotic and probiotic compounds, for example, from fruit, vegetables, and yogurt. These recommendations establish a framework that is good for both your body and the little microscopic organisms with which it coexists. **LPI**

Do Probiotics Affect Bowel Regularity?

According to the Activia® website (Activia.us.com), the product makers state: "Clinical studies show that Activia® with *B. Lactis* DN 173-010 helps with slow intestinal transit or occasional irregularity when consumed 3 times per day for 2 weeks as part of a balanced diet and healthy lifestyle."

A 2011 systematic review and meta-analysis of 11 clinical trials with 464 participants reported that probiotic supplementation has a modest effect on reducing bowel transit time, mainly in the elderly and those with initial slow transit time. *B. Lactis* DN 173-010 (three trials, 139 participants) and *B. Lactis* HN019 (two trials) were deemed to have the most significant treatment effects.

Yogurt is an excellent package of nutrients, and the regular consumption of yogurt has been associated with many positive health effects, such as improved cardiovascular health and successful weight management. The presence of pro- and prebiotics in yogurt may even mediate these and other health effects; scientists are still gathering information. The possibility of reduced transit time may be yet another reason to consume yogurt, but the benefit depends on the frequency of consumption and type of probiotics.



In Memoriam: Dr. Emile Zuckerkandl



Dr. Emile Zuckerkandl, former President of the Linus Pauling Institute of Science and Medicine, died at home in Palo Alto, California, on November 9, 2013, at the age of 91.

Zuckerkandl was born in Vienna, Austria, in 1922. His mother was a painter and his father was a biochemist, and his grandparents were involved in anatomy, psychoanalysis, and the arts. Zuckerkandl was forced to flee Austria during WWII, but thanks to help from Albert Einstein, immigrated to the United States to continue his education.

Zuckerkandl received a master's degree in physiology in 1947 from the University of Illinois and a doctorate in biology from the Sorbonne in Paris. He worked initially at the Marine Biological Laboratory of Roscoff in Brittany on hemocyanin, a copper-containing oxygen transport protein found in the blood of invertebrates like mollusks and crustaceans.

In 1959 Dr. Linus Pauling offered Zuckerkandl a post-doctoral fellowship in his lab at the California Institute of Technology in Pasadena and persuaded him to study hemoglobin, the iron-containing oxygen transport protein in humans, instead of hemocyanin. Pauling had been interested in hemoglobin for many decades, including his discovery of abnormal hemoglobin as the cause of sickle-cell anemia—the first disease to be described as a molecular disease—and studies that discovered the alpha-helix, a main structural theme of proteins. In the 1950s, Pauling became increasingly interested in evolutionary theory, stimulated by his interest in possible genetic mutations caused by exposure to radioactive fallout. Under Pauling's direction, Zuckerkandl began applying electrophoretic and chromatographic techniques to study differences in hemoglobin among primates. This collaboration resulted in a number of important papers, including the 1962 seminal paper, "Molecular disease, evolution, and genetic heterogeneity," that introduced the concept of the molecular clock, which was specifically named as such in another paper in 1965. In this new field of molecular evolution that Pauling and Zuckerkandl also called "chemical paleogenetics," the molecular clock was invoked to calculate and time the divergence of species based on the assumption of a fairly constant rate of mutation (amino acid substitutions, coded by DNA) and comparisons of the amino acid makeup of hemoglobin among species. This paper played a crucial role in the origin of the field of molecular evolution, and Zuckerkandl later became the founding editor of *The Journal of Molecular Evolution*.

In 1964 Zuckerkandl returned to France for many years, where he founded a molecular biology research institute, the Research Center of Macromolecular Biology, in Montpellier. He spent 1976 working on gene regulation at the Marine

Biological Laboratory in Wood's Hole, Massachusetts, followed by a stint as a visiting Professor at the University of Delaware. At Pauling's invitation, Zuckerkandl joined the Linus Pauling Institute of Science and Medicine in Menlo Park, California, in 1977 and was elected by its Board of Trustees as President and Director in 1980. Under Zuckerkandl's guidance, the Institute expanded into new research areas, including molecular evolution, aging, virology, genetics, and gene regulation in cancer, while continuing strong programs in orthomolecular medicine. After his retirement from the Linus Pauling Institute of Science and Medicine in 1992, he founded the Institute of Molecular Medical Sciences in Palo Alto to carry out research in molecular biology and genetics.

On May 29, 1998, Rick Hicks, former vice president of the Linus Pauling Institute of Science and Medicine and a long-time colleague of Zuckerkandl and Pauling, hosted a small dinner on the occasion of the Austrian government's award of the Austrian Cross of Honour for Science and



Dr. Emile Zuckerkandl and Dr. Linus Pauling

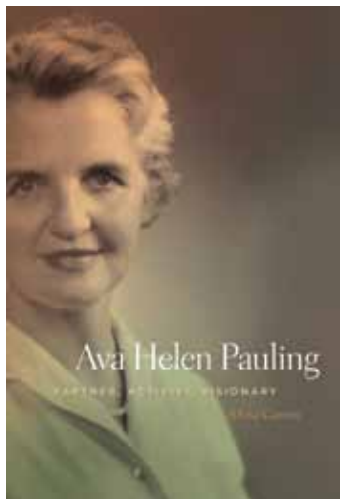
Art to Zuckerkandl. Among the guests were Margrit and Robert Mondavi and Nobel Laureates Edward Lewis of Caltech and Henry Taube of Stanford.

The Linus Pauling Institute inducted Zuckerkandl into the Linus Pauling Institute Society in May, 2007, at the Diet and Optimum Health Conference in Portland, Oregon. Zuckerkandl was honored for his "extraordinary vision and generosity," qualities that sustained and enhanced the Institute in California for many years, and for his great personal sacrifices on behalf of the Institute.

Stephen Lawson, LPI's administrative officer and former CEO of the Linus Pauling Institute of Science and Medicine, knew Zuckerkandl for many years and co-directed the Laboratory for Research in Gene Regulation with him. Lawson remembers Zuckerkandl as a brilliant scientist, accomplished pianist, humanitarian, and exceedingly kind and generous person with catholic interests spanning diverse sciences and the arts.

Emile Zuckerkandl is survived by Jane, his wife for over 65 years. **LPI**

New Biography of Ava Helen Pauling



Ava Helen Pauling: Partner, Activist, Visionary by Dr. Mina Carson—the only biography of Linus Pauling’s wife, Ava Helen—was published by OSU Press in 2013. Dr. Carson, an expert on American social and cultural history, is an associate professor of history at Oregon State University.

Linus Pauling met Ava Helen Miller at their alma mater, Oregon Agricultural College—now Oregon State University. They married in 1923 when Pauling was a graduate student at the California Institute of Technology in Pasadena and had four children. Ava Pauling was interested in social and political causes and persuaded her husband to devote more time to educating the public about the dangers of radioactive fallout from the atmospheric testing of atomic bombs. Their joint efforts, including circulating worldwide a petition among notable scientists, were instrumental in the test ban treaty of 1963 to end the atmospheric and underwater testing of atomic bombs, signed by the U.S.S.R., the United States, and the United Kingdom. In 1963, Pauling won the Nobel Peace Prize for 1962 in recognition of his important role in securing the treaty. He often said that he thought his wife should have shared the award. Among the treasured items in the Ava Helen and Linus Pauling Papers at OSU is a trove of love letters that Linus wrote to Ava Helen over decades. [LPI](#)



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Developments

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These gifts aren’t overly complicated. You transfer cash, stocks, real estate or other property to a trust designed to pay an income to you (and/or someone of your choosing) for life or for a specified term of years. When the trust ends, the remaining assets pass to the Oregon State University Foundation to support research in the Linus Pauling Institute.

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For more information about how these flexible gifts can benefit you and your family, please contact LPI’s gift-planning specialists at the OSU Foundation. There is no obligation and, of course, your request will be respected as strictly confidential. If you have any questions or would like a confidential illustration on how a life-income gift can help support LPI research meaningful to you, please contact Jeff Comfort, Vice President, Principal Gifts and Gift Planning, OSU Foundation at 800-354-7281 or jeff.comfort@oregonstate.edu. [LPI](#)



Linus Pauling Institute

Stephen Lawson, Research Newsletter Editor

Oregon State University
307 Linus Pauling Science Center
Corvallis, Oregon 97331

phone: 541-737-5075
fax: 541-737-5077
email: lpi@oregonstate.edu
website: lpi.oregonstate.edu



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