

# LINUS PAULING INSTITUTE

OREGON STATE UNIVERSITY  
RESEARCH NEWSLETTER

SPRING–SUMMER 2018

Research Update on ALS Clinical Trials with Copper ATSM. **LOOK INSIDE** ►

## EMBRACING BOTANICAL DIETARY SUPPLEMENTS

**BOTANICAL SUPPLEMENT USE IS ON THE RISE, BUT SAFETY ISSUES REMAIN**

By **Richard B. van Breemen** and **Ruth Muchiri, Ph.D.**

In addition to nutrients, plants synthesize a vast array of metabolites that we call phytochemicals. Since many phytochemicals have pharmacological activities, plants are the original source of medicines for treating disease or maintaining human health.

Today, many of the synthetic (or semisynthetic) drugs that we use regularly still find their origins in plants. Although pharmaceuticals now replace many plant-based traditional medicines, **botanical supplements** (see sidebar on page 5) are a primary source of health products for a large part of the global population. In several cases, they remain a viable alternative to drugs.

And it is safe to say that the demand for botanical products is increasing. The worldwide

market for botanical dietary supplements exceeded \$33 billion in 2010 and is projected to surpass \$140 billion by 2024.

Despite their prevalence and popularity, there is much to learn about the beneficial properties of botanical products, as well as the potential drawbacks of their use. This is the focus of the van Breemen laboratory.

Our laboratory has decades of experience studying a variety of natural products. Beginning in the 1990's, we were part of an international team of scientists and nutritionists conducting human studies of the bioavailability and bioconversion of provitamin A carotenoids like beta-carotene. These molecules, naturally produced by plants, can be converted by the body

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Oregon State University  
Linus Pauling Institute

# FROM THE DIRECTOR



**Richard van Breemen, Ph.D.**

The new director of the  
Linus Pauling Institute

**Is there something you  
would like to see in the  
next LPI newsletter?**

Let us know!

Email [lp@oregonstate.edu](mailto:lp@oregonstate.edu)

or call [541-737-5075](tel:541-737-5075)

At the end of 2017, after packing my laboratory and household in Chicago onto several moving vans during a snowy Illinois winter, my wife Denise Hynes and I boarded a flight headed west. We were ready for our new adventures in Oregon and at Oregon State University. Ever since, we have been enjoying the much milder climate of the Willamette Valley and the beautiful OSU campus.

Leading the Linus Pauling Institute is an incredible opportunity, and I am humbled to have been selected for this great honor. Having met Dr. Pauling after he gave a seminar during my first year as an assistant professor of chemistry at North Carolina State, I think of how amazed I would have been to glimpse into the future. I now lead the Institute that he founded.

I had followed Dr. Pauling's research concerning dietary supplements for years before I met him, sharing in the belief that vitamins and other dietary supplements can enhance health and prevent disease. We also shared a passion for promoting peace. In our brief conversation, Dr. Pauling and I talked about the process of writing scientific manuscripts and approaches to teaching undergraduate chemistry. It was over 30 years ago now, but I wish we had more time in our conversation to discuss his beloved Institute.

Although dedicated to research, the Linus Pauling Institute has always provided an exceptional environment for educating the next generation of experts on micronutrients and dietary supplements as they relate to maintaining human health and preventing disease. All of our principal investigators are professors or associate professors at Oregon State University and maintain active research programs. Undergraduates, graduate students, postdoctoral fellows, and visiting scholars are found in every program. The LPI is the perfect environment to promote the education of brilliant young minds.

Many of my own graduate students completed their Ph.D. dissertations before leaving the University of Illinois, but five remain in my laboratory at the LPI: Emily Rue, Luying Chen, Jialin Liu, Alan Wong,

and Danial Nosal. I would like to thank Kerry McPhail and Theresa Filtz for helping my students transfer to the Department of Pharmaceutical Sciences in OSU's College of Pharmacy and to resume course work immediately.

To enhance support for graduate education in natural products research, my first grant application since arriving at OSU was a predoctoral training grant to the NIH National Center for Complementary and Integrative Health. I would like to thank my co-principal investigator, Taifo Mahmud in the College of Pharmacy, for all his help making this possible. If successful, this will provide funding for additional students at OSU to complete their Ph.D. programs in the LPI.

Ruth Muchiri, a postdoctoral trainee who worked with me in Chicago, has now joined the ranks of the LPI. Ruth is now part of the research staff, helping to manage our research laboratory.

Our group complements others within the LPI, not just by adding new expertise, but because of our history of a collaborative approach. We have been an integral part of NIH-funded research centers for over three decades, including centers dedicated to the discovery of mechanisms of cancer chemoprevention by natural products and the discovery of natural products with pharmacological activities.

Currently, we are working with the Center for Botanical Dietary Supplements that I co-founded while working in Chicago with the late Norman Farnsworth. This center is focused on the study of the safety and efficacy of botanical dietary supplements for women's health. This experience will guide the development of collaborative research teams within the LPI and other experts at Oregon State University, supported by new external center grants.

All the members of my research team are pioneers in the development and application of mass spectrometry-based methods for natural product drug discovery (see cover article). We are also experts in human metabolism and bioavailability of many compounds from natural products.





Dr. van Breemen strongly supports increased training opportunities for all students in the LPI's research laboratories. Pictured with Dr. van Breemen is Danial Nosal, a graduate student in his laboratory.

Continuing to rely heavily on biomedical mass spectrometry, I will work toward developing strong relationships with manufacturers of instrumentation for scientific research and establishing new centers of excellence that will benefit not only the LPI but also researchers across the Oregon State University campus.

My thanks go to the generosity of the many friends of Linus Pauling and the LPI, alumni of OSU, the support of University President Ed Ray, and the tireless effort of the OSU Foundation. Because of you, the Institute is located in the beautiful and state-of-the-art research building, the Linus Pauling Science Center (LPSC).

The facilities of the LPSC are designed to support translational research. This includes high-end chemistry instrumentation like mass spectrometry and nuclear magnetic resonance spectroscopy, research animal facilities, and core laboratories dedicated to the implementation and successful execution of human clinical trials.

The building is also home to Ava's Cafe on the first floor (named after Ava Helen Pauling, wife to Linus; she also attended OSU). The art enriching the Linus Pauling Science Center includes colorful light sculptures by Steven Knapp, paintings based on the crystal structure of vitamin C by Bill Shumway, amazing lenticular art by Chris Dean, and recently, due to the generosity of Joahn H. Facey, a collection of paintings by Gershon Benjamin.

All told, I certainly enjoy the privilege of working at the LPI.

I would like a moment to thank Dr. Fred Stevens for his phenomenal effort as interim director of the Linus Pauling Institute. As you know, following the retirement of director Balz Frei, Fred volunteered his services. During the 18 months that followed, Fred reorganized the core laboratories of the LPI to improve efficiency, replaced retiring administrative staff, encouraged the LPI faculty to submit grant applications, and succeeded in expanding external support for the LPI from the

National Institutes of Health.

Fred also helped recruit a new LPI principal investigator, Melissa Haendel, who joined OSU this spring (more about Melissa and her research program coming in the next newsletter). Therefore, all my gratitude goes to Fred for continuing the LPI on the path to success.

I look forward to working with Fred, Melissa, and all of the faculty, students, and staff at the LPI as we continue and expand our research into understanding how micronutrients and natural products can help all of us live healthier and longer lives.

Sincerely,

Richard B. van Breemen, Ph.D.  
Linus Pauling Endowed Chair and Director  
Linus Pauling Institute

to vitamin A when needed. Until recently, there was very little information on how efficiently people process the carotenoids from fruit and vegetable sources.

With highly specific tracer studies, we revealed that the body has an inherent inefficiency in the conversion of plant carotenoids to vitamin A. These findings resulted in changes to how many regulatory agencies throughout the world now calculate the amount of bioavailable vitamin A from plant sources.

More recently, we co-founded the first NIH-funded center for botanical dietary supplements research. At that point, the focus of our laboratory shifted to investigating the safety and efficacy of botanical supplements, with the philosophy that consumers everywhere should have access to botanical dietary supplements backed by quality scientific evidence.

Thus, we support a stepwise approach to achieving this goal: mandate quality assurance, determine a mechanism of action, enact standardization, and conduct clinical studies.

The need for this approach may not be immediately apparent. Throughout history, issues have plagued botanical supplements. A frequent problem was contamination, either by heavy metals, microbes, or pesticides. Misidentification of plant species was also common, producing supplements that contained little to none of the compounds intended to provide health benefits.

For too long, regulations on botanical supplements have been lax. Because of this, we have advocated that botanical dietary supplements should be produced using best practices and evidence-backed techniques. In 2007, the US Food and Drug Administration (FDA) implemented a regulation requiring current Good Manufacturing Practice to be used in the production of all dietary supplements marketed in the United States. Although this has made a significant contribution to the safety of botanical supplements, issues remain.

Consistent with the mission of the LPI, our laboratory seeks to promote optimal health by understanding uses of botanical products. By determining the mechanisms of action for these dietary supplements, their roles in maintaining health and preventing disease can have a strong scientific foundation.

This process includes identifying active constituents, such as a compound or a group of compounds that has a desired effect. After doing so, it allows standardization of supplements both chemically and biologically. Although many approaches are available for the chemical standardization of a botanical dietary supplement, we typically rely on mass spectrometry-based (MS) assays.

Indeed, our laboratory has pioneered the use of mass spectrometry for the identification of active compounds in botanical dietary supplements and other complex mixtures of natural products, such as microbial and fungal extracts.

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**“Invented over a century ago to prove the existence of isotopes of the elements, biomedical mass spectrometry has become the fastest and most sensitive analytical tool for determining both identities and amounts of compounds.”**

**—Richard van Breemen, Ph.D.**

Key to our success is advancing the state-of-the-art quantitative analysis of natural products.

The screening assays that we employ start with an extraction of the active compound from the mixture. This often involves a chemical binding reaction of the molecule to a protein or other target macromolecules. Once extracted, the compound of interest can be analyzed by employing mass spectrometric characterization.

One of these approaches is called Pulsed UltraFiltration (or PUF) mass spectrometry. Compounds binding to cellular protein receptors are separated from unbound compounds using filtration techniques. Thus, only the compounds that interact with cell proteins are saved for further analysis.

A similar approach, called MagMASS (Magnetic Microbead Affinity Selection Screening), tethers cell receptors to microscopic magnetic particles. This facilitates the separation of the bound components of a natural product simply by applying a magnetic field. These separated constituents are identified by MS to precisely define which compounds have biological activity.

Once active botanical compounds are identified, the next step for our laboratory has always been to apply well-established techniques typically employed in drug development to determine how well compounds are absorbed and metabolized by the body.

Some of these assays utilize a human colon cell line as a mimic of the intestinal wall. These cells can be used to estimate intestinal permeability and transport, predicting how much of the compound could reach the blood stream.

Other assays address metabolic stability and conversion to active, inactive, or toxic compounds by enzymes. If compounds are modified by drug metabolizing enzymes found in the liver, for example, the information can be used to predict possible interactions that may occur with conventional prescription drugs.

To ensure that these laboratory studies are as relevant to human health as possible, it is important to conduct these experiments not only with the active compounds we have isolated but also with mixtures of compounds that represent what is found in a given dietary supplement.

However, these assays are all performed in a laboratory. Ultimately, addressing issues of safety and efficacy in botanical



dietary supplements requires properly designed trials with human volunteers. Such studies begin with Phase I clinical trials specifically designed to explore issues of safety. They also provide valuable information regarding the bioavailability and pharmacokinetics of the active constituents as well as possible drug-botanical interactions.

Phase II clinical trials are the next step in the process. These studies are generally longer and use more participants. While Phase II trials provide additional safety data, the primary purpose is to measure outcomes on health or the progress toward treatment of a disease. Thus, these are the first studies to address efficacy, whether or not the supplement can work as intended.

Our laboratory has carried out five Phase I clinical trials and three Phase II clinical trials of botanical dietary supplements. This includes trials on black cohosh (*Actea racemosa*), red clover (*Trifolium pratense*), hops (*Humulus lupulus*), and lycopene (commonly found in tomato).

These studies were all made possible by utilizing the speed, sensitivity, and accuracy of MS-based methods on the cutting edge of scientific discovery.

Consumers of botanical dietary supplements expect that the products they use today are safe and effective. Our laboratory is setting the standard for rigorous preclinical and clinical scientific study to achieve these goals. Studies of botanical dietary supplements and educating young investigators about natural product research will continue to be the focus of the van Breemen lab at the Linus Pauling Institute.

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## What is a botanical supplement?

A botanical is a plant or, more often, a part of a plant that is valued for its therapeutic properties. Botanical products are usually prepared in some sort of easy-to-use form, but people use botanical products for many different reasons and in different ways. Today, we can find botanical extracts in things we eat, put on our skin, or use to clean our clothes.

**Botanical dietary supplements** are botanical products intended for consumption along with your everyday diet. A large variety of forms are available: Teas, tinctures, extracts, powders, and tablets are the most common.

Because many plants can be included in botanical supplements, it should be stressed that the nutrients and chemical metabolites (referred to as phytochemicals) inside them can be equally varied. Indeed, plants used for producing botanicals can contain different compounds depending on the varieties used or how they are grown.

This also means that some botanical products can contain compounds that are considered beneficial alongside others that are not. This presents challenges in determining how well they perform their intended purpose (efficacy) or how much harm they can cause (safety). These are now important areas of research at the LPI.

Although the analysis of a whole botanical supplement is important, it can have many overlapping effects. Often, researchers test a class of compounds, or one specific molecule, isolated from plants to determine its function.

Recent examples of this type of work from the LPI include the research conducted by Dr. Fred Stevens on xanthohumol (a chalcone isolated from the hops plant) and by Dr. Emily Ho on sulforaphane (an isothiocyanate typically found in cruciferous vegetables).

# GIVING AGING CELLS A NEEDED BOOST

WHEN CELLS PRODUCE THEIR OWN ANTIOXIDANTS, IT HELPS THEM RESIST STRESS.

Despite the outward steps we take every day to live longer and feel better as we age, biochemical changes occurring 'behind the scenes' can undermine those efforts. Researchers from the Linus Pauling Institute – especially those in the Healthy Aging Program – are investigating new ways to help keep us as healthy as possible as we get older.

In advanced age, our bodies have more trouble dealing with the stresses of everyday life. This is not in reference to anxiety-provoking events that occur throughout your day but to stresses on a cellular level. Cells encounter potentially dangerous molecules all of the time and are constantly at risk of developing permanent damage.

What are the sources of these harmful compounds? Unfortunately, you don't need to look further than your medicine cabinet. Pharmaceutical drugs and over the counter medicines that are designed to keep us healthy can also do us harm, especially when mixed together in a number of different ways. Although cells naturally have protective mechanisms, these start to fall apart with time, leaving us vulnerable to injury as we get older.

Tory Hagen, Ph.D., the Helen P. Rumbel Professor for Healthy Aging Research and Director of the Healthy Aging Program at the Linus Pauling Institute, is leading a team of researchers to investigate ways to help older cells resist stress. For decades, Dr. Hagen has studied a specific detoxification compound produced by cells called glutathione, which helps them resist a variety of toxic stresses. The problem is that glutathione levels decline with age, setting the stage for a wide range of age-related health problems.

Studies by Dr. Hagen's group have shown that there may be new strategies to help older cells boost their glutathione levels. Before going into details of their findings, including their paper published in 2016 (see **References**), it may be helpful to give a short primer on glutathione: what it is and how it acts in the body.

Glutathione is produced by cells from three amino acids: cysteine, glycine, and glutamate. The cysteine in the molecule is particularly important because it makes glutathione a very reactive molecule.

One role of glutathione is to bind to reactive oxygen species, preventing them from reacting with cell proteins or DNA. This makes glutathione one of the cell's most important antioxidant defenses.

Cells also use glutathione to bind to toxins and other foreign compounds they might encounter. This immediately limits the toxin's potency and hastens its removal from the body.

**“...glutathione is such a vital antioxidant that its existence appears to date back as far as life on this planet began to utilize oxygen...”**

**–Tory Hagen, Ph.D.**

“We've known for some time of the importance of glutathione. It is a strong antioxidant and detoxicant,” explains Dr. Hagen, “In fact, glutathione is such a vital antioxidant that its existence appears to date back as far as life on this planet began to utilize oxygen – about 1.5 billion years. It's a principal compound to detoxify air pollutants, heavy metals, pharmaceuticals, and many other toxic insults.”

Dr. Hagen was one of the first in the world to characterize that glutathione – and the associated detoxification it provides – declines with age. This loss of glutathione presents a particular issue for older adults, especially those who take multiple pharmaceutical drugs. If these compounds aren't removed from the body fast enough, they can cause lasting damage.

Failures in these detoxification pathways have links to cardiovascular disease, type 2 diabetes, and cancer.

This brings us back to the most recent work by Nicholas Thomas, Ph.D., a postdoctoral researcher working with Dr. Hagen. To explore the roles of glutathione in the body, Dr. Thomas compared the resistance of animals of varying ages to the effects of a molecule called menadione.

Drs. Hagen and Thomas use menadione as a model toxin, probing not one but a variety of cellular dextoxification pathways. Watching the response of liver cells to menadione provides them with a model of how the liver would respond to encountering any toxin. In this case, cells use glutathione to defend themselves from the harmful effects of menadione, ideally preventing any damage from occurring.

Their results show that cells isolated from young animals can meet a menadione challenge. Although it initially dropped, the cells maintained their glutathione concentrations at about 40% of their initial levels.

However, liver cells isolated from old animals quickly demonstrated the extent to which age affected them: After menadione was added, glutathione levels dropped rapidly from already low concentrations. After 5 minutes of exposure to the toxin, only about 10% of the glutathione remained in these cells.

“Our work shows how cells from younger animals are far more resistant to stress than those from older animals,” says Hagen, “When placed under the same stress, older cells were quickly depleted of glutathione and died.”

In their more recent studies, the Hagen lab took this work a step further and investigated the impact of glutathione depletion on mitochondria. Mitochondria are cellular organelles involved in energy production and other roles critical to overall cell function. Mitochondria are also seen as a critical linchpin for changes in





Mitochondria are not only the major source of energy production in our cells, but they can also determine many aspects of how well we age. However, maintaining glutathione levels in the mitochondria is vitally important in all stages of life.

cells that are observed with age.

Although most (~75%) of a cell's glutathione is found in the cytosol – the fluid that fills the cell – mitochondria contain much of the rest, more than any other cellular organelle. Glutathione synthesis occurs only in the cytosol, but the mitochondria can accumulate and maintain its own share of this antioxidant.

Therefore, it is possible that you can deplete the glutathione present in the mitochondria without markedly affecting the rest of the cell, and vice versa. So if cells are losing glutathione in response to menadione, does it affect mitochondria to the same degree?

This was the focus of the next set of studies by Dr. Thomas. He found that exposing the whole cell to menadione also depleted glutathione levels in mitochondria. Like the rest of the cell, mitochondria isolated from older animals had dramatically lower glutathione levels after the menadione was added.

Although these data are in preliminary stages, it seems that there are other consequences to this loss in glutathione. There are indications that mitochondria

from older animals exposed to menadione show a decline in energy production, a phenomenon not observed in cells from young animals.

Overall, the core problem is that when cells or mitochondria have low levels of glutathione, they aren't able to adequately protect themselves. To address this, Dr. Thomas administered a compound called N-acetyl-cysteine (or NAC) to cells to help maintain glutathione levels.

It is known that the cysteine provided by NAC can be funneled into the cell's glutathione synthesis machinery, but they wanted to know if NAC provided before a challenge would be an effective prophylactic against exposure to a toxin.

The results of their studies are clear: pre-treatment with NAC increased glutathione levels in the older cells and largely helped offset the degree of cell death. It is not yet clear what NAC supplementation does to glutathione levels in the mitochondria.

NAC is currently in use in hospitals: In emergency medicine, it is given to patients in a toxic crisis, such as ingestion of heavy metals. The results so far show NAC to

be a safe compound, even at extremely high levels, at least in the short-term.

Dr. Hagen is optimistic: "Using NAC might improve the metabolic resilience that we're naturally losing with age."

Dr. Hagen believes that low levels of a NAC supplement as a prophylactic, instead of an intervention may allow glutathione levels to be maintained for detoxification in older adults. The uses of NAC are not just limited to the elderly: The detoxification potential offered by glutathione could apply to prescription drugs, cancer chemotherapies, and treatment of other health issues.

However, these applications will require additional research in human subjects. For now, the Hagen lab is working on other ways of bolstering the stress response of older adults, at least on a cellular level.

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Dr. Adrian Gombart focuses his research on understanding the role of vitamin D in the immune system. Pictured with Dr. Gombart is his graduate student, Yang Zhang.

## VITAMIN D-INFUSED BANDAGES:

### PROVIDING VITAMIN D INSIDE A WOUND IS A NOVEL APPROACH TO FIGHTING POST-SURGICAL INFECTION

**With the help of new technology, researchers at the Linus Pauling Institute are developing nanofiber “vitamin D bandages” that may cut the risk of post-surgical infections.**

Working with collaborators at the University of Nebraska Medical Center, Dr. Adrian Gombart, a principal investigator at the LPI, is investigating the use of nanofiber wound dressings. These are no ordinary bandages. Created by a process called electrospinning, the dressings are made of very thin fibers that can incorporate molecules like vitamins or drugs. In Dr. Gombart’s case, the fibers are impregnated with vitamin D.

Vitamin D is useful in a number of ways when it comes to wound healing. It can reduce the amount of inflammation caused by an overactive immune system to improve the healing process (see sidebar). Vitamin D can also help fight against microbial infections, primarily by stimulating immune cells to produce the

anti-microbial peptide called cathelicidin.

In theory, the nanofibers would provide vitamin D to immune cells gathering at a surgical site or a wound, allowing the immune system to destroy the invading bacteria before infection can take hold. Since these dressings are dissolvable, there is no need for a repeat surgery to remove them.

“The materials we are developing will hopefully be used in surgical patients to reduce the possible risk of a variety of infections from drug-resistant bacteria,” Gombart said. “Vitamin D is safe, and the bandages simply harness the host’s own immune response to fight infection directly, in a manner that antibiotic-resistant pathogens are less likely to defend against.”

Surgical site infections are not only common but also very costly to treat, especially when antibiotic-resistant bacteria like MRSA are involved. Unlike antibiotics, which target specific enzymes found in bacterial cells in order to weaken them, anti-microbial peptides work by a mechanical action on bacterial cell membranes. They either burrow into the bacterial membrane to make a pore (or hole) or they cover the membrane to disrupt it. Either action makes it easier for other aspects of the immune system to kill the offending microbe.

“Antibiotic compounds typically represent a single point of attack on bacterial pathogens,” says Gombart, “One reason we’re pursuing this strategy of vitamin D release is that an activated immune system



provides multiple points of attack.”

Two recent studies by the Gombart lab, Dr. Arup Indra (adjunct faculty member of the LPI), and their collaborators from Nebraska Medical Center have explored how these vitamin D dressings will work in patients.

The first study, published in late 2017, focuses on the use of nanofibers containing 25-hydroxyvitamin D<sub>3</sub> – the form of vitamin D found circulating in the blood. This molecule is formed in the liver, following vitamin D consumption (from foods or supplements) or synthesized in the skin after exposure to UV light from the sun.

Through exposing isolated skin cells or immune cells to the sutures containing 25-hydroxyvitamin D nanofibers, Dr. Gombart’s team observed a continuous release of the molecule. As they expected, this was followed by the production of anti-microbial peptides.

When immune cells are activated, more of these peptides are produced, suggesting that a vitamin D conversion process was necessary for full activation. This produces 1,25-dihydroxyvitamin D, a form of the vitamin so potent that the body only converts it from 25-hydroxyvitamin D when needed.

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### One of the problems of using laboratory mice in a vitamin D study is that their immune cells do not make anti-microbial peptide in response to vitamin D.

In their recently published study, the team decided to work with dressings that contain only the active 1,25-dihydroxy form of the vitamin. In cell models, it worked just as well as the fibers used in the previous study containing the 25-hydroxy form.

In addition, they took one step closer to getting it into human patients by employing an animal model. “The work in our first paper was completely in cell culture,” Gombart explained, “Cells don’t always act the same way in isolation as they do in the body. So the next step was

testing the dressings in animals.”

Moving into animal models presents a unique challenge. One of the problems of using laboratory mice in a vitamin D study is that their immune cells do not make anti-microbial peptide in response to vitamin D. In fact, this is an adaptation found only in humans and other primates.

Therefore, Dr. Gombart routinely uses a genetically modified mouse model where specific portions of the human cathelicidin gene are inserted into the mouse genome. This allows vitamin D to control the production of the anti-microbial peptide, as it does in humans.

To make use of this animal model, they gave the animals a surgical wound in their skin under controlled conditions to minimize the animal’s discomfort. After wounding, a sterile nanofiber disc containing 1,25-dihydroxyvitamin D was placed inside the wound. As expected, cathelicidin production increased near the wound site but not in skin that had no wound or in the wound without 1,25-dihydroxyvitamin D.

Their colleagues in Nebraska repeated the experiments in human skin samples obtained from people undergoing plastic surgery, finding similar results.

Together, this shows a great advance in the application of vitamin D. While their latest studies did not look at risk of infection or measure rate of wound healing, this is planned for the future. While they clearly saw benefits of the bandages in their animal trials at the site of a wound, it is unclear how a sustained release of 1,25-dihydroxyvitamin D might affect other tissues.

Gombart and his collaborators currently have funding from the National Institutes of Health to continue their animal trials, looking at different aspects of the vitamin D dressings in order to bring them safely into human trials.

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## Vitamin D and Immunity

The active form of vitamin D regulates the expression of many genes and is now recognized to be a potent modulator of the immune system. The vitamin D receptor is expressed in several types of immune cells. Human macrophages (one type of immune cell) can convert vitamin D to its active form where the cells become activated, targeting the immune response.

Vitamin D regulates adaptive immunity, which involves antibody reactions and certain inflammatory responses. For instance, vitamin D inhibits a class of T helper cells (called Th<sub>1</sub> cells) and therefore may limit inflammatory responses that might damage healthy tissue. On the other hand, vitamin D stimulates regulatory T cells, which may have a role in preventing autoimmune disease or complications due to organ transplants.

Despite the relationship between low vitamin D status and autoimmune disease, few randomized controlled trials have been conducted. Vitamin D is currently under investigation by a number of laboratories across the world; evidence from clinical trials is forthcoming.

For more information on vitamin D or other micronutrients in immunity, see the Micronutrient Information Center: [lpi.oregonstate.edu/mic/health-disease/immunity](http://lpi.oregonstate.edu/mic/health-disease/immunity)

# MICRONUTRIENTS FOR BONE HEALTH

## FROM THE MICRONUTRIENT INFORMATION CENTER

By **Victoria J. Drake, Ph.D.** and **Barbara Delage, Ph.D.**

### INTRODUCTION

The skeleton serves as a mineral storehouse for the body, primarily a reserve for calcium and phosphorus. While it may seem like an inert structure, the skeleton is made of tissues and cells that are continually active throughout a lifetime. Bones grow in size and shape during childhood, adolescence, and early adulthood, but replacement of old bone tissue by new bone tissue – a process called ‘remodeling’ – occurs throughout life. The remodeling phase consists of a constant process of bone resorption (breakdown) and formation, replacing old bone that is brittle or damaged with new bone. This also can release calcium and phosphorus into the circulation when they are needed by the body.

Beginning around age 34, the rate of bone resorption exceeds that of formation, leading to an inevitable loss of bone mass with age. If bone mass falls to a critically low level, bone softening (osteomalacia), pre-osteoporosis (osteopenia), osteoporosis, and bone fracture can occur.

Nutrition and physical activity have important roles in building bone mass during childhood and young adulthood and slowing bone mineral loss later in life. Calcium and vitamin D play a prominent role in bone health throughout life, but other micronutrients, including magnesium, fluoride, and vitamins A, B, C, and K, are also important for producing and maintaining healthy bone.

Two types of bone cells are involved in maintaining mineral balance in bone. **Osteoclasts** dissolve bone, liberating minerals into the blood stream. **Osteoblasts** build the bone matrix, depositing minerals onto collagen proteins. If mineral levels in the body are lacking, the resulting bone will be low in mineral density.

### CALCIUM

Calcium is a major structural element in bones and teeth; in fact, 99% of calcium in the body is found here in the form of hydroxyapatite, a molecule made of calcium and phosphate. Calcium is also found in blood and soft tissues where it is critical to body functions like muscle contraction, nerve impulse conduction, or constriction and relaxation of blood vessels.

The body tightly regulates blood calcium concentrations. When intake of calcium is low, calcium is leached from bone to maintain normal calcium concentrations in blood; therefore, adequate dietary calcium is critical to limit bone resorption (demineralization) and preserve skeletal integrity and health.

Chronically low calcium intake in childhood and early adulthood prevents bone from reaching optimal mineral mass and will increase the risks for osteoporosis and bone fracture in older age. Low calcium intake in the adult years can accelerate the decline in bone mineral density.

There are many food sources of calcium, including dairy and fortified food. Calcium intake from food is preferred over supplements, since the safety of long-term calcium supplement use has not been adequately evaluated. Total intake of calcium should not exceed 2,500 mg/day in adults 19-50 years, and 2,000 mg/day in adults over 50 years.

There is currently no consensus regarding the use of calcium supplements to prevent fractures in older adults.

### VITAMIN D

An important function of vitamin D is to control blood concentrations of calcium and phosphorus in order to preserve normal physiological function. Very severe vitamin D deficiency causes rickets in children and osteomalacia in adults.

Vitamin D is found naturally only in a few foods. Exposure to sunlight is the primary source of vitamin D; however, the use of sunscreen effectively blocks vitamin D production in the skin. Additionally, geographical location, season, and time spent outdoors affect vitamin D synthesis in the skin. For example, sunny days in the winter at northern latitudes produce no vitamin D in the skin, even when going outside at midday when UV light is at its strongest.

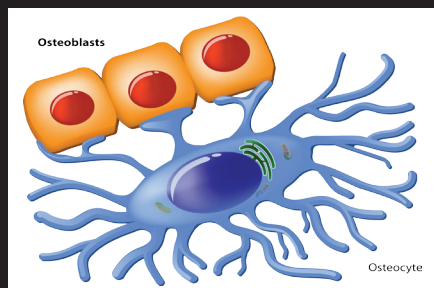
Supplementation may be needed in children and adults to reach recommended levels – the American Academy of Pediatrics recommends 400 IU/day of supplemental vitamin D for all infants, children, and adolescents. The Linus Pauling Institute recommends that all adults take 2,000 IU/day of vitamin D. Physicians can perform blood tests to determine if your vitamin D status is low.

Intervention studies in older adults have found little-to-no effect of vitamin D supplementation alone on fracture risk, but some studies have found that combined vitamin D and calcium supplementation reduces fall or fracture risk.

### PHOSPHORUS

Along with calcium, phosphorus is a major structural component of bone in hydroxyapatite crystals. Although phosphorus deficiency can lead to rickets and bone softening, it is uncommon in healthy people.

High phosphorus intakes – which are quite common in the US – have not been associated with an increased risk of osteoporosis. However, low calcium intakes





combined with high phosphorus intakes represent a serious risk to bone health.

MAGNESIUM

About 60% of the body’s magnesium is found in bone, where it influences the size and strength of the hydroxyapatite crystals. The effect of magnesium inadequacy on bone health in humans is poorly understood. Higher magnesium intakes may be linked to a lower risk of poor vitamin D status, but the evidence is currently too limited to suggest that magnesium supplementation could reduce risk of osteoporosis or fracture.

OTHER MICRONUTRIENTS

Many other micronutrients have essential roles in bone health, including fluoride, vitamin A, some B vitamins, vitamin C, and vitamin K. However, there is little evidence to suggest that supplementation with these micronutrients above current recommendations provides further benefit.

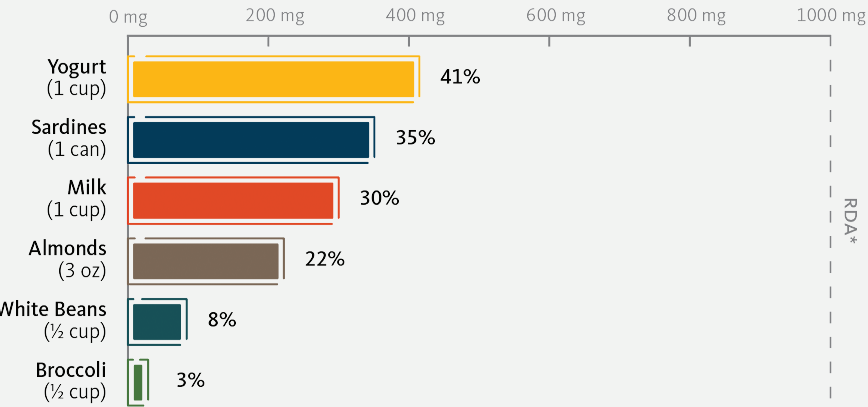
Striving to reduce phosphorus and sodium intakes while increasing intakes of potassium (e.g., by eliminating processed food and increasing intake of fruit and vegetables) is likely to indirectly benefit bone health through improving overall health. In addition, physical activity is highly beneficial to bone health during development and throughout life. Any amount of physical activity, even in frail elderly people, is better than none and will bring some benefits to bone health.

References:

[lpi.oregonstate.edu/mic/health-disease/bone-health](http://lpi.oregonstate.edu/mic/health-disease/bone-health)  
Heaney, RP. *Bone biology in health and disease*. Modern Nutrition in Health and Disease. 11<sup>th</sup> ed: Lippincott Williams & Wilkins (2014) ISBN-10: 1605474614  
Weaver, CM et al., Osteoporos Int. 27 (2016) doi: 10.1007/s00198-015-3440-3

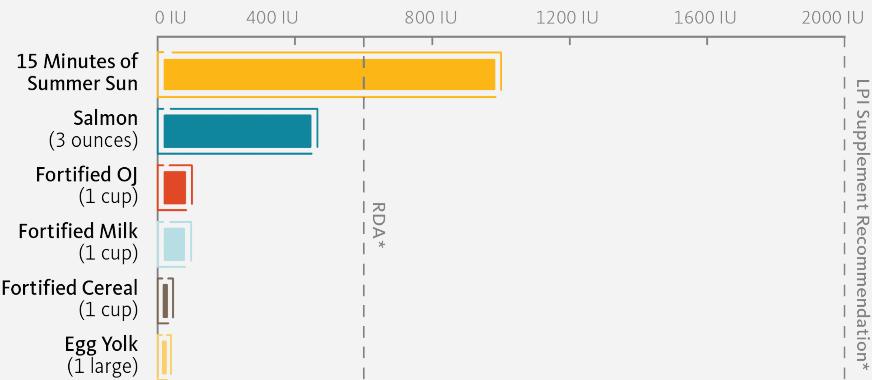
CALCIUM

There are varied food sources of calcium, though the highest amounts are often found in dairy products. Plants provide dietary calcium, but it is less bioavailable in plants with high levels of oxalate or phytate. Calcium intakes of 1,000 mg/day (total from diet and supplements) should cover the needs of most adults. The RDA is higher for adolescents (1,300 mg/day), older women (>50 years, 1,200 mg/day), and older men (>70 years, 1,200 mg/day).



VITAMIN D

Food is a poor source of vitamin D. Vitamin D production by sun exposure can vary by season, latitude, and skin type. Therefore, supplements are often recommended. The Linus Pauling Institute recommends that generally healthy adults take 2,000 IU (50 µg) of vitamin D from supplements daily. LPI also recommends daily intakes (total from diet and supplements) of 400 to 1,000 IU for infants and 600 to 1,000 IU for children and adolescents.





## AN UPDATE ON COPPER-ATSM CLINICAL TRIALS

**Superoxide dismutase (SOD) is a protein that transforms a reactive form of oxygen called superoxide into relatively harmless hydrogen peroxide. In the development of Amyotrophic Lateral Sclerosis (ALS), this might all change. With a genetic mutation or with the loss of an important mineral in the protein, SOD can start to harm motor neurons.**

Dr. Joseph Beckman's work at the Linus Pauling Institute has focused around understanding the role of SOD in the development of ALS. Because ALS is a disease that results in progressive loss of motor neurons in the spinal cord, it is

difficult to target and treat. However, a breakthrough came in 2016.

With Dr. Blaine Roberts (Beckman's former graduate student) and their collaborators from Australia, Dr. Beckman's group published his results with a molecule called copper-ATSM. Later that year, we introduced their findings in this newsletter: *copper-ATSM increases the lifespan of mice genetically engineered to contract ALS*.

It was far from a cure for the disease, but it was a start. In a field where progress is very difficult to achieve, their results seemed miraculous. Yet, a lot of questions

remained. Primarily, since the studies had been completed in mice, would copper-ATSM work in people with ALS?

Despite these limitations, the response from the ALS community has been enthusiastic. Needless to say, many people who are currently living with the disease are desperate for treatment, because diagnosis is usually considered a death sentence.

Since the work on copper-ATSM is progressing, we thought it would be a good time to provide you with a short update by way of answering a few frequently asked questions on this topic.

### CAN COPPER REALLY HELP ALS?

Linus Pauling advocated the importance of the right micronutrient at the right time and in the right place to promote health and prevent disease. In animal models of ALS, one of the problems was that the SOD protein was missing a mineral – it could either be zinc or copper, as they both were important to enzyme function. Yet SOD deficient in just one of these metals was very toxic to motor neurons.

Recently, Dr. Beckman and his colleagues began to focus on restoring copper to SOD, hoping to make the protein functional again. Although copper was important, giving animals more of the mineral alone did not help restore the protein. This is because their approach was untargeted: copper went to every part of the body. Eventually the large amounts of copper became harmful to the animals.

The special chemical carrier called ATSM was key to their success. The ATSM backbone provided a shield around the copper atom, one that would only come off in specific tissues of the body. Effectively, ATSM delivered copper to the spinal cord and prevented other tissues from experiencing the negative effects of excess copper.



## WHAT IS THE STATE OF CU-ATSM CLINICAL TRIALS?

Copper-ATSM is already used in people for the imaging of hypoxic tissues. Before Dr. Beckman and his collaborators finished their trial in animals, it was not considered a treatment of ALS. Because of it was not approved for this use, clinical trials must be conducted to test it for that purpose.

To develop this compound as a treatment for disease, special clinical trials are needed to evaluate safety and efficacy. Although the trials on safety are nearly concluded and the results are promising, the truth is that many ALS drugs do not get past this critical step.

**Phase I clinical trials** are the first step in the process of new drug development. These studies test for safety, starting with small doses of the drug to determine any side effects that would indicate toxicity. As the trial progresses, subjects take increasing doses of copper-ATSM to determine how much poses a risk to health.

Once the Phase I trial is complete, researchers can begin testing for efficacy. This comes in the form of **Phase II and III clinical trials** that are designed to determine if a particular dose of the drug can slow or stop the progression of ALS.

Procypra is a company that is taking the lead in developing copper-ATSM for treatment of Parkinson's disease and ALS. Their Phase I clinical trial is nearly complete, and it can be found on ClinicalTrials.gov under the identifier, NCT02870634. The first results from this clinical trial show that copper-ATSM appears to be safe even at the projected therapeutic doses. However, many hurdles lie ahead before it can be available to many ALS patients.

ALS families question the need for such trials. Time is very valuable, especially for those with the diagnosis of a terminal disease. **The risks in taking an unproven treatment are very serious.** The wrong amount, timing, or route of administration of the drug, combined with issues of drug purity, metabolism, and interactions, have the potential of killing someone within a matter of days.

## WHAT ABOUT OTHER ALS DRUGS?

Copper-ATSM has a long way to go before general approval by the FDA. However, two other drugs are much closer to approval for the treatment of ALS.

**Masitinib** is a tyrosine kinase inhibitor that is designed to reduce some of the effects of chronic inflammation in cells. Much of the preclinical work for Masitinib was done here at the LPI in collaboration with the Pasteur Institute of Montevideo Director, Dr. Luis Barbeito. Masitinib is currently being used with other drugs in Phase III clinical trials to find its efficacy in ALS.

**Edaravone** (also called Radicava) is a synthetic free radical scavenger (antioxidant) that reduces protein nitration, a particular problem in the progression of ALS. Edaravone was recently approved for use in the United States since it appears to slow the progression of the disease. Edaravone is currently only available as an intravenous infusion.

More drugs are also in development. Progress towards the treatment of ALS has been accelerating. Although drugs like copper-ATSM are going to clinical trials faster than ever before, it is still a slow process. Now that there has been some success in treating this awful disease, more substantive successes are just around the corner.

## WHAT ABOUT THE FUTURE OF ALS RESEARCH AT LPI?

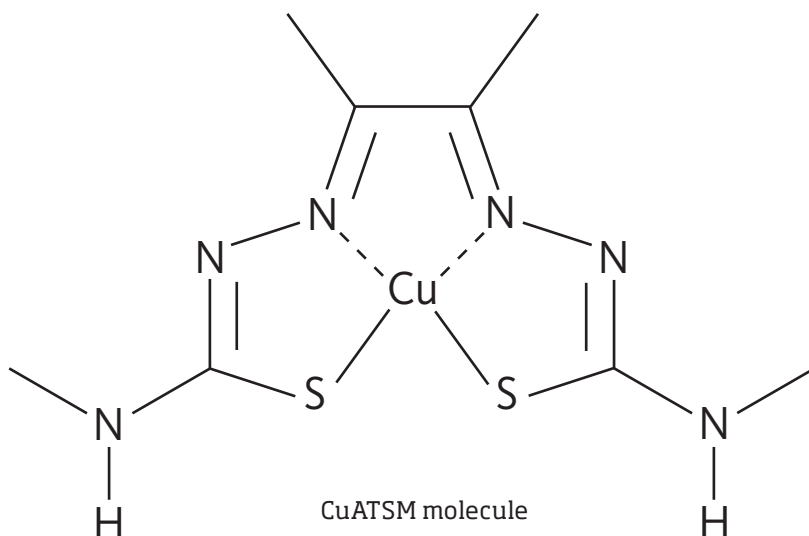
Dr. Beckman's group and his collaborators continue to work on understanding the underlying causes of ALS. Their research has identified multiple targets for therapy – pointing the way to the next generation of therapies for the treatment of ALS.

For example, they are testing different compounds based on the structure of copper-ATSM that may work on multiple forms of ALS and other neurodegenerative diseases. Novel compounds are being tested in ALS models to see if they, too, can prevent or delay the onset of disease. Also, the Beckman group continues working on ways to improve the efficacy of existing drugs like Edaravone and copper-ATSM.

We know that many people around the world are watching these studies carefully, and we will bring you more updates from Dr. Beckman's lab as soon as they become available.

For more information on copper-ATSM, clinical trials, or the current state of ALS research at the Institute, we encourage you to email us at [lpi@oregonstate.edu](mailto:lpi@oregonstate.edu)

The copper-ATSM molecule (shown below) has one atom of copper protected by a ring of carbon, sulfur, and nitrogen. Originally developed to image tumors, it can also deliver copper to hypoxic tissues like the spinal cord.



# LPI STUDENTS



## Isabelle Logan

Ph.D. Student, Department of Biochemistry and Biophysics

Born and raised in Belgium, Isabelle became the first person in her family to make the leap to the United States, where she made her new home in Oregon. First in her family to earn a degree in higher education, she graduated *summa cum laude* from Portland State University with a bachelor's degree in Chemistry. Upon graduation, she took the opportunity to come to OSU to continue her education in science.

During her first year as a graduate student, Isabelle not only found a passion for teaching but also found her place at the bench. She joined the lab of Dr. Adrian Gombart, principal investigator at the LPI. In conjunction with Dr. Stevens' laboratory, Isabelle studies xanthohumol and its derivatives as potential anticancer agents. She is also working to establish animal models to test the effects of xanthohumol on gut health.

Isabelle's goal after she completes her dissertation is a postdoctoral position at the Fred Hutchinson Cancer Research Center in Seattle. She is passionate about stimulating young students' interests in entering STEM fields. Isabelle was selected as the 2017-18 Christopher and Catherine Mathews Graduate Fellow in the Department of Biochemistry and Biophysics and has received the Franz Stenzel M.D. and Kathryn Stenzel II Scholarship for two years running.



## Brian Head

Ph.D. Student, Department of Molecular and Cell Biology

Originally from southern California, Brian came to OSU with an interest in animal medicine. He obtained a bachelor's degree in Zoology here in 2014. As an undergraduate, Brian had many experiences in laboratories across campus, which kindled an interest in research. This led him to complete an M.S. in Animal Science, also from OSU.

After his master's work, Brian spent some time working at the LPI with Dr. Maret Traber on projects related to vitamin E. Not long after, Brian started the Ph.D. program under the guidance of Dr. Traber. His current project focuses on the genetic regulatory networks involved in vitamin E deficiency in zebrafish, especially how lower vitamin E levels may alter embryonic development.

Brian is also interested in the effects of vitamin E in the aging brain and determining if vitamin E can play a role in preventing neurodegenerative disorders like Alzheimer's disease. Beyond graduate school, Brian plans to advance knowledge of nutritional adequacy during development and aging in his own laboratory, incorporating molecular techniques with bioinformatics. He is currently supported by the Provost's Distinguished Graduate Fellowship, and he was recently awarded the Mark Spontenburgh Endowed Graduate Fellowship through the LPI.



## Amanda Kelley

Ph.D. Student, Department of Biochemistry and Biophysics

Growing up only a mile away from Yellowstone National Park, Amanda found herself wanting to know more about the diversity of organisms living in such harsh conditions. Her curiosity about life at the molecular level led her to Montana State University where she received a bachelor's degree in Chemistry. Positive research and teaching experiences, along with a desire to know more about human health, brought her to explore more of the scientific world in graduate school.

Because of interests in mitochondrial function and healthy aging, she is now working with the LPI's resident expert on aging, Dr. Tory Hagen. She is examining the changes that occur in the mitochondria of a mouse model of Alzheimer's disease.

Shortly after coming to LPI, Amanda entered the IGERT (Integrative Graduate Education and Research Traineeship) program in Aging Sciences, with funding from the National Science Foundation. The training program's focus on interdisciplinary projects allowed her to learn about the relationship between systolic blood pressure and dog ownership in older adults. Amanda was the previous recipient of the Mark Spontenburgh Endowed Graduate Fellowship at the LPI and also received a travel award to attend the American Aging Association conference, giving her much appreciated support for her work.

# OUTREACH

As part of the LPI's public outreach mission, the **Healthy Youth Program (HYP)** empowers youth and families to achieve optimal health through hands-on education. The HYP is currently cultivating partnerships with a local hospital and medical school to execute the Culinary Health Education and Fitness (C.H.E.F.) Project, which aims to build a culture of health in local Oregon counties. This includes annual training of medical students to help implement dozens of youth and family cooking classes in rural communities.

In Corvallis, the HYP summer work and internship program for high school students is gearing up for their fourth year in the school gardens. This year, weekly 'Farm Stand' events feature distribution of fresh fruit and vegetables for physician-referred families through a partnership with the Benton County Health Department. This program is supported

by the OHSU Knight Cancer Institute Community Partnership Program.

Since its debut in 2000, the LPI's **Micronutrient Information Center (MIC)** has provided evidence-based, expert-reviewed information on the roles of vitamins, minerals, and other dietary factors in health and disease. This free website ([lpi.oregonstate.edu/mic](http://lpi.oregonstate.edu/mic)) has a global audience of more than one million people. Some articles are available in Spanish, and Japanese translations are underway.

New content is available on the topics of bone health, osteoporosis, and micronutrient inadequacies; recent updates include articles on fruit and vegetables; the B-vitamin, niacin; and coenzyme Q10.

We would like to thank ChromaDex for their support and Pfizer for awarding us two grants. Many thanks to our readers who have generously supported the MIC!



A group from the Healthy Youth Program's C.H.E.F. Project cooking class practice their knife skills as they prepare a recipe together.

# DEVELOPMENTS

Things have been quite busy at LPI! The arrival of Dr. van Breemen has brought with it lots of new faces, activity, and equipment to LPI labs. With the arrival of his team comes many brilliant young scientists, hard at work advancing the Institute's research. They remind us all of the impact that a public research institution can have on the development of a young person's career.

We have also been very fortunate to recruit Dr. Melissa Haendel as our new Director of Translational Data Science. She brings tremendous expertise in biomedical data analytics that can be applied to both basic science research and translational clinical work. Her expertise will allow our researchers to access, interpret, and incorporate large data sets across multiple disciplines in incredibly powerful ways.

Nothing excites researchers more than "multidisciplinary collaborations," and the whole Institute is buzzing with new ways that our strengths can be leveraged to advance the work of the Institute. This is truly an exciting time to be at LPI – new leadership, researchers, and technologies have set the stage for a new era of breakthroughs at the LPI.

All of us at LPI want to thank the many loyal supporters who have kept the wind in our sails throughout the years. You have been integral partners in our success. We hope that you will stay engaged with our work as we continue to advance Dr. Pauling's legacy of research and discovery.

Sincerely,

Amanto Marcotulli, J.D.  
OSU Foundation  
[OSUFoundation@osufoundation.org](mailto:OSUFoundation@osufoundation.org)

## GIVE TO THE LPI

There are many ways that you can support the research and education missions of the LPI - Contact the OSU Foundation today!

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Dr. Richard van Breemen, Director of  
the Linus Pauling Institute, describes  
his work on botanicals. **LOOK INSIDE** ►



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