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INTRODUCING THE TISLab

AN INTERDISCIPLINARY TEAM THAT FOCUSES ON DATA INTEROPERABILITY TO EXPEDITE TRANSLATIONAL RESEARCH

By **Melissa Haendel, PhD** and **Julie McMurry, MPH**

Dr. Melissa Haendel recently joined Oregon State University as the Director of Translational Data Science at the LPI. Her multidisciplinary research team is investigating new ways to utilize a wealth of scientific data across the world to help explain, diagnose, and prevent disease. Here, Dr. Haendel and the team's Associate Director, Julie McMurry, give an overview of their laboratory and the powerful techniques they have developed.

Despite the great lengths that researchers undertake to publish their findings, the greatest challenge is applying that knowledge to the prevention or treatment of disease.

Although personalized healthcare holds great promise, simply collecting vast amounts of data about patients is not adequate. Even the most meticulous description of a single patient is uninterpretable without millions of comparisons to other people or to research in cells and animals. Medical data need to be interpreted in the light of the dynamic interplay between demographics and environment, which include dietary choices and exercise.

Optimizing the use of complex data sets is the challenge faced by Dr. Haendel's laboratory, known as the Translational and Integrative Sciences Laboratory (or the TISLab). We want to know how we can navigate the vast landscape of highly specific scientific knowledge and apply it in a way that helps people live longer, better lives.

The TISLab's philosophy is one of collaborative innovation among researchers, clinicians, and patients across the globe. Our multidisciplinary

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FROM THE DIRECTOR

Richard van Breemen, PhD
Director
Linus Pauling Institute

Linus Pauling's fascination with micronutrients was an extension of his ideas surrounding the molecular basis of disease. Convinced that optimum doses of these small molecules (vitamins and minerals) might help prevent disease and enable people to live longer, healthier lives, he advocated for dietary supplementation of many of these molecules.

To facilitate this research, in 1973 Pauling founded a research facility that would come to be known as the **Linus Pauling Institute of Science and Medicine**. As new evidence accumulated concerning the benefits of taking vitamin supplements, many followed his lead. However, there was also a great deal of controversy emerging concerning the use of dietary supplements.

In the early seventies, the U.S. Food and Drug Administration (FDA) proposed new guidelines on dietary supplements. The FDA was interested in limiting the levels of vitamins in supplements, treating dietary supplements as drugs, and imposing a strict set of regulations for their production and use.

An advocate for taking large amounts of certain micronutrients, particularly vitamin C, Dr. Pauling opposed the FDA's plan. Specifically, he disagreed with proposed restrictions on the amounts of vitamins in dietary supplements.

The main concerns were that the regulations proposed by the FDA would have likely increased the cost of dietary supplements and would have limited their availability to consumers.

Always an advocate for the people, Pauling campaigned to preserve public access to dietary supplements. As public outcry increased, Congress began hearings to consider these regulations when Linus Pauling volunteered to testify.

"I am interested in medicine and all of its aspects, and in particular in preventive medicine..." Pauling stated in testimony before U.S. Senate subcommittees in 1973 and 1974, "I think to classify vitamin C and other vitamins even as over-the-counter drugs would have a serious effect on the health of the American people."

Concerned that much of the research on vitamins and minerals was inadequate to inform the FDA's decision. Pauling wrote in a prepared statement to Sen. Edward Kennedy, Sub-Committee on Public Health in August 1974, "No serious consideration whatever has been given to the question of optimum daily intake, the amount that leads to the best of health." It is a question researchers are still trying to answer, even 45 five years later.

Pauling supported some regulations aimed to help consumers. "I advocate some controls over the sale and advertising of vitamins, but not through classifying them as drugs," Pauling stated. "...The proposed FDA regulations do not in my opinion establish an effective mechanism for protecting the consumer against the abuses of misrepresentation..."

At the time of Dr. Pauling's testimony, support for his position came in the form of tens of thousands of letters, telegrams, and telephone calls to Congress. Ultimately, the will of the people prevailed.

In 1976, the Vitamin-Mineral Amendment prohibited the FDA from limiting the potency of vitamin or mineral supplements. If a supplement made claims to treat or prevent disease, the FDA could still regulate these products and investigate product claims. Otherwise, these supplements – including high-potency products – **could not be treated as drugs**.

However, the fight over dietary supplements did not end there. In 1990, the Nutrition Labeling and Education Act, which enacted the modern nutrition facts panels we find on food products, extended supplement regulations to include botanical dietary supplements. This law provided directives to the FDA to implement new rules for establishing the validity of health claims of all dietary supplements.

From 1993 to 1994, Congressional hearings were held again to determine how dietary supplements should be regulated. New bills were drafted, and passionate arguments were presented for and against placing

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The current members of the Linus Pauling Institute, pictured outside the Linus Pauling Science Center at Oregon State University.

limits on the FDA regulation of dietary supplements.

In October of 1993, less than a year before his death, Linus Pauling again entered into the discussion regarding the fate of dietary supplements. Submitting a letter to the U.S. Senate Hearing on Dietary Supplements, he again advocated for appropriate regulation that did not block access of the people to supplements.

In support of a new **Dietary Supplement Health Education Act** (often referred to as DSHEA), Pauling wrote, "In the 1970s, I testified before Congress on legislation that would help protect consumers against vitamin fraud and set the stage for better education... It is imperative to maintain the highest quality of purity, safety, and performance in regard to consumer products and services..."

Pauling applauded the bill because it directed the National Institutes of Health to support research on the safety and efficacy of supplements. "Decade after decade, century after century, the world has gotten better, largely because of scientific discoveries. The challenge is for government to keep up with scientific progress."

DSHEA was made into law in 1994 (Public Law 103-417). In effect, it replaced or amended the previous laws by providing an all-encompassing definition for dietary supplements. Under DSHEA, the category of dietary supplements now includes vitamins, minerals, herbs or other botanicals, and amino acids, including extracts or combinations of these ingredients.

DSHEA authorizes the FDA to require the use of current Good Manufacturing Practice in the production of dietary supplements, which has contributed substantially to consumer safety. This requirement is, in part, the realization of Pauling's plea for dietary supplements of "the highest quality of purity, safety, and performance."

Unlike previous legislation, DSHEA included an important research and educational component. It created the Office of Dietary Supplements within the National Institutes of Health, which was also endorsed by Dr. Pauling. The mission of the Office of Dietary Supplements is much like that of the Linus Pauling Institute, namely, to explore the role of dietary supplements in maintaining health and preventing chronic disease. It also promotes scientific study by coordinating funding.

DSHEA has had many positive effects on the science behind dietary supplements. For example, the Office of Dietary Supplements initiated an extramural botanical center program in 1999 to examine the effects of botanical dietary supplements. I co-founded the first of these academic botanical centers with the late Norman Farnsworth in Chicago, and served as the director of this NIH-funded center until moving to Oregon.

Today, my laboratory at the LPI continues to carry out translational clinical studies and education on the safety and efficacy of botanical dietary supplements through this program.

Earlier this year, a member of Congress telephoned to ask my opinion whether stricter regulation of dietary supplements should be implemented. I responded as I hope Pauling would have, that existing regulations regarding safety and accurate labeling are adequate but require better enforcement, and that research and education regarding the safety and efficacy of botanical dietary supplements need more vigorous support.

DSHEA encompassed many of the important issues in the field of dietary supplements that had been supported by Dr. Pauling and were embodied into the mission of the Linus Pauling Institute. Established more than 20 years before DSHEA, the Linus Pauling Institute remains committed to determining optimal levels of dietary supplements for maintaining health and preventing disease, as well as training new generations of experts in this field.

Sincerely,

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

Continued from cover — **The TISLab**

team of biologists, bioinformaticians, clinicians, and computer scientists are the experts on the approaches that translate basic research into precision healthcare.

The tools that the TISLab creates are sophisticated computer algorithms designed to sort through vast quantities of data. At the heart of these programs are logical constructs called **ontologies**. Loosely defined as search criteria for computers to sort and classify data, ontologies offer a way to distill and connect information from many different sources without a loss of meaning.

How does this work? At a basic level, ontologies are structured vocabularies that define relationships among concepts. You can think of it as a type of instruction manual for how computers can piece together disaggregated data on different topics from a variety of sources.

Say that you want to search the scientific literature related to Alzheimer's disease. A search based solely on just the name of the disease might miss a highly relevant result – especially those using the term 'dementia'. Alternatively, searches regarding particular genes important in to the development of Alzheimer's disease might yield very specific information about a protein and less about its roles in the disease as a whole.

While similarities in search terms might be obvious to a person, the computer executing the query does not have this type of generalized knowledge. However, it is not practicable for humans to peruse and hand pick every result, so ontologies train the computer to think with the nuance of a human but the precision of a machine.

Ontologies do this by expanding any given term to its synonyms and other related terms. This will ensure that relevant results are not missed just because one term within the family is missing.

Ontologies can help integrate existing data using much the same principles. Biomedical data are constantly being generated throughout the world, existing in various formats and contexts. Once we disassemble these sources into more granular, fundamental components, we can use ontologies to weave them together again.

The applications of this technology are diverse. The TISlab's bioinformatics technologies and diagnostic software packages are designed primarily to help clinicians and researchers translating basic science to its applications in human health.

Our flagship effort, the Monarch Initiative, is a project designed to help reveal the genetic causes of certain rare conditions. This is because obscure connections between basic research and symptoms are often used to tease apart the underlying causes **(see sidebar on Page 5)**. Currently, the tools we have developed are in use by many organizations worldwide, such as the National Institutes of Health and Genomics England.

Loosely defined as search criteria for computers to sort and classify data, ontologies also offer a way to distill and connect information from many different sources without a loss of meaning.

Although we are developing several tools geared solely for use by clinical researchers and healthcare professionals, some of the tools within the TISLab involve interactions with patients. Funded by the **Patient Centered Outcomes Research Institute (PCORI)**, we seek to develop tools that utilize the power of our ontologies.

Unlike standard patient surveys which are either superficial or focused on a particular disease, ontology-driven tools can be open ended, allowing patients to quickly describe their conditions comprehensively without wading through thousands of questions that do not apply to them.

While a physician's judgment will always remain central to medical decisions, ontology-driven tools provide a broad foundation for the interpretation of medical data and pave the way for the use of multiple data sources in creating precision treatment plans.

The goal is to have the patients provide the key data that will support a collaboration with their clinicians. Take, for example, a patient who goes to see a cardiologist about heart problems, a sleep specialist about sleep apnea, and to a podiatrist about hammer-toe. Later, he or she winds up an emergency room with spontaneous collapsed lung. The patient may be unaware that these various symptoms have an underlying common cause.

Especially if this person only reports a partial symptom profile to each physician, any one of them might easily overlook the possibility that the patient has Marfan syndrome (a rare genetic disease that has been proposed as the explanation for Abraham Lincoln's unusual stature and "long, pendulous" arms). While a clinician may have seen hundreds of cases of diabetes, she may only see a single case of a particular rare disease in her entire career.

Ontology-driven representations of medical knowledge can help guide physicians to ask the most relevant questions and draw clearer connections between symptoms and diseases, thereby arriving more quickly and reliably at the best course of action, whether it is surgery, or changes in dietary habits. Leveraging ontologies to unify the large amount of research data from other organisms makes logical leaps possible.

Lastly, the TISLab is also involved in the **Biomedical Data Translator**, funded by the National Institute of Health's National Center of Advancing Translational Sciences (NCATS). The goal of this program is to integrate many different data types across basic and clinical data sources to classify diseases. This classification can improve the potential for drug design. It also may allow certain drugs to be repurposed, leading to novel disease treatment strategies.

This type of classification can apply to different types of interventions simply by expanding this approach beyond its original goals. As part of our work with the Linus Pauling Institute, we plan on using the same tools developed in the Biomedical Data Translator to detect drug-nutrient interactions.

The advent of massive data and affordable high-capacity computing ushers in the opportunity for unprecedented discovery by association. Increasingly, causal reasoning that links clinical and basic science data together is yielding diagnostic and therapeutic insights. We believe ontologies will be a game changer that will have a profound impact on clinicians and the way they practice medicine.

All members of the TISLab are grateful for the very warm welcome we have received at Oregon State University. Our team, which includes researchers from different research institutions around Oregon, hopes to leverage the expertise available at all of these academic centers to accelerate translational science research.

Many of our projects would not be possible without the help of our collaborators around the world.

More information on our initiatives, team members, and findings, see our website at **http://tislab.org**.

For those interested in the Monarch Initiative and its work on rare genetic conditions, see **https://monarchinitiative.org**.

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DIAGNOSTIC USES OF ONTOLOGIES

Ontologies are particularly useful in identifying rare genetic disorders – diseases so infrequent that there may be only a handful of individuals affected by them at a particular time. In these cases, the medical documentation is typically sparse, and symptoms of the disease may vary widely among individual carriers.

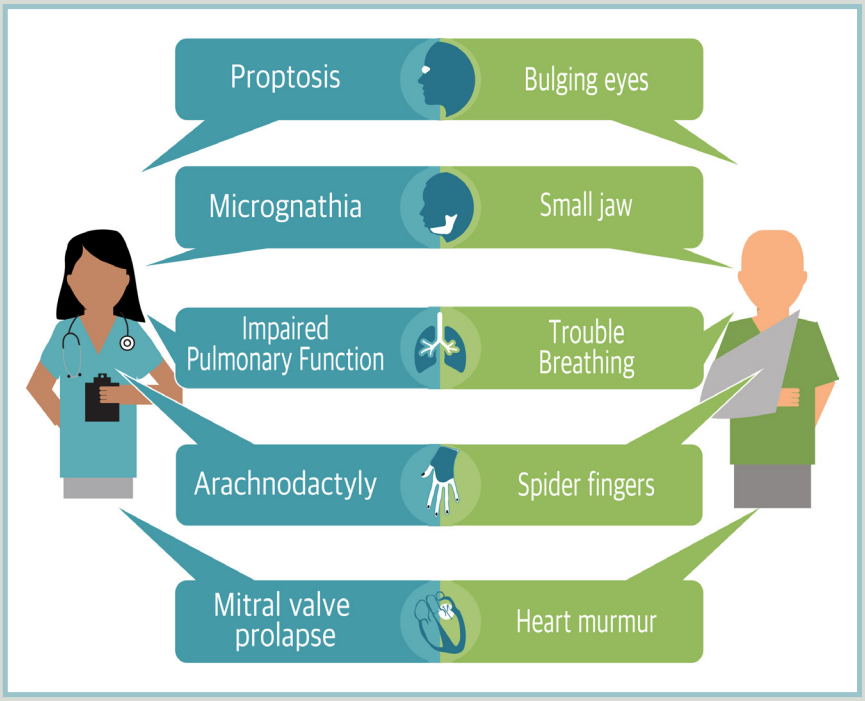
The rarity of the condition contributes to a poor understanding of how the condition will present itself. Diagnostic criteria for disease are established by how often the symptoms appear in disease carriers. When only one person carries a genetic disease, there is no way of knowing if all the associated symptoms will appear in any other individual with that condition.

Ontologies can bridge diagnostic gaps in several ways. First, by assembling data from a diverse number of sources, ontologies connect clinical observations to data collected in animals and cells. Comparing effects of a genetic change across different model systems may reveal some patterns.

Second, ontologies often use fuzzy logic systems to search data. They expand their criteria beyond exact symptom matches, allowing connections even if a disease presents differently from previous reports.

Lastly, ontologies can connect plain language descriptions to medical conditions (see the Figure below). This can serve as a 'patient translator' of sorts, allowing individual accounts of physical abnormalities or symptoms to be treated the same as medical data.

Ontologies can piece together medical and research data to provide insights into many diseases. As we move into an era of personalized healthcare, these tools may someday help us better understand our own relationship with health and disease.



Dr. Fred Stevens, the resident expert on xanthohumol at the Linus Pauling Institute, and one of his graduate students, Ines Paraiso.

IMPROVING ON HOPS

XANTHOHUMOL DERIVATIVES IN METABOLIC SYNDROME

Xanthohumol shows promise in the treatment of metabolic syndrome, but it can form a potent phytoestrogen when metabolized. Sponsored in part by the Buhler-Wang Research Fund at the OSU Foundation, researchers from Dr. Fred Stevens' laboratory have developed novel derivatives of this compound that show promise in laboratory animals.

Found in small quantities in beer and some extracts like tea, xanthohumol is a flavonoid isolated from the hops plant. Based on evidence from pre-clinical studies that show beneficial effects on energy metabolism, this compound might be useful in treating or mitigating obesity.

Dr. Fred Stevens, principal investigator at the Linus Pauling Institute and Professor of Pharmaceutical Sciences in the College of Pharmacy, has been studying the effects of xanthohumol for over two decades. In his studies in animals, xanthohumol can normalize blood glucose and cholesterol levels and reduce inflammation.

The bottom line is that xanthohumol appears to be helpful in the fight against **metabolic syndrome**.

More a cluster of symptoms than a disease, medical doctors consider metabolic syndrome as a combination of the following: abdominal obesity, high blood pressure, high blood sugar, low levels of "good" cholesterol, and high levels of triglycerides. It is also marked by high levels of inflammation.

Together, these metabolic issues are a serious condition and a major risk factor for type 2 diabetes and cardiovascular disease, as well as being associated with cognitive dysfunction and dementia.

Although metabolic syndrome is rarely mentioned in the news, chances are you know someone who has it. More than one-third of U.S. adults have this condition, and it is being increasingly diagnosed in children and adolescents.

Dr. Stevens believes that human clinical trials with xanthohumol could demonstrate its effectiveness against metabolic syndrome. However, there is one catch: taking xanthohumol can have some unwanted side effects.

"The metabolism of xanthohumol produces a very potent phytoestrogen," he explains, "This effectively limits the potential for widespread use. If someone took hops extracts for long periods of time, for example, estrogen-like effects could outweigh the benefits."

Phytoestrogens are compounds that mimic the structure of estrogen. Found in plants, these compounds can stimulate estrogen responses in humans. Although low exposures to phytoestrogens are not necessarily detrimental, some phytoestrogens are more potent than others.

Gut microorganisms can transform xanthohumol into 8-prenylnaringenin, one

of the most potent phytoestrogens found in nature. This makes taking xanthohumol a risky venture, as the estrogenic potential of 8-prenylnaringenin could contribute to the progression of endometriosis, breast cancer, male infertility, or prostate cancer growth.

Dr. Stevens reasoned there must be a way to 'fix' xanthohumol. As a trained pharmacist, he approached the problem from the perspective of its chemistry: "A single double bond in the xanthohumol molecule is responsible for the side reaction to 8-prenylnaringenin," explains Dr. Stevens, "If we can get rid of it, the metabolite would not be formed."

It was a plausible solution. Selective hydrogenation would result in compounds similar to xanthohumol but without the potential for estrogenic properties. In other words, it might preserve the characteristics that make it so valuable in metabolic syndrome while removing the potential for side effects.

In the end, the Stevens laboratory was able to create two hydrogenated derivatives of xanthohumol **(see illustration)**. Neither of these new compounds contain the double bond that facilitated further metabolism.

The Stevens laboratory along with collaborators at Oregon Health & Science University (OHSU) demonstrated their approach in a recently published journal article. After administration to mice, the new derivatives improved blood glucose levels, insulin response, and sensitivity to leptin, a hormone that signals for satiety and regulates energy expenditure.

Moreover, testing showed that the new xanthohumol derivatives appear to be more effective than the original compound without the formation of any estrogenic metabolites.

"Tetrahydroxanthohumol is especially potent in the mice fed a high-fat diet," said Dr. Cristobal Miranda, an associate professor working with Dr. Stevens and intimately involved in this research. "The animals fed this compound had the least body weight gain during the trial."

Typically, mice that eat a high-fat diet perform poorly in cognitive testing. The more fat they consume, the worse their memory and ability to focus. Drs. Stevens and Raber (a professor of behavioral neuroscience at OHSU), found that the new derivatives improved spatial learning and memory compared to controls.

These findings could also be important for people suffering from cognitive impairments associated with changes in diet and gut bacteria, but further studies are needed to support this hypothesis.

Effectively, xanthohumol derivatives counteract some of the effects of the poor diet, including liver toxicity normally seen with higher amounts of fat.

"It is plausible that the hydrogenated derivatives are more bioavailable than xanthohumol – that would explain why they work better," Stevens added. "Despite altering their metabolic fate, these compounds appear to have low liver toxicity and no intrinsic estrogenicity, which suggests they are not causing a toxic response."

More experiments are needed before these compounds are ready for human trials. One open question is the dosing in humans required to obtain the same beneficial response observed in animal models. However, the Stevens laboratory is up for the challenge. Having pursued xanthohumol this far, they are not going to rest until this research on metabolic syndrome finds a place in the medical community.

Hopsteiner, Inc. and the OSU Foundation's Buhler-Wang Research Fund supported this research. The Stevens laboratory now has research funding from the National Institutes of Health for testing xanthohumol in people with Crohn's disease and studying its effects in animal models of Alzheimer's disease.

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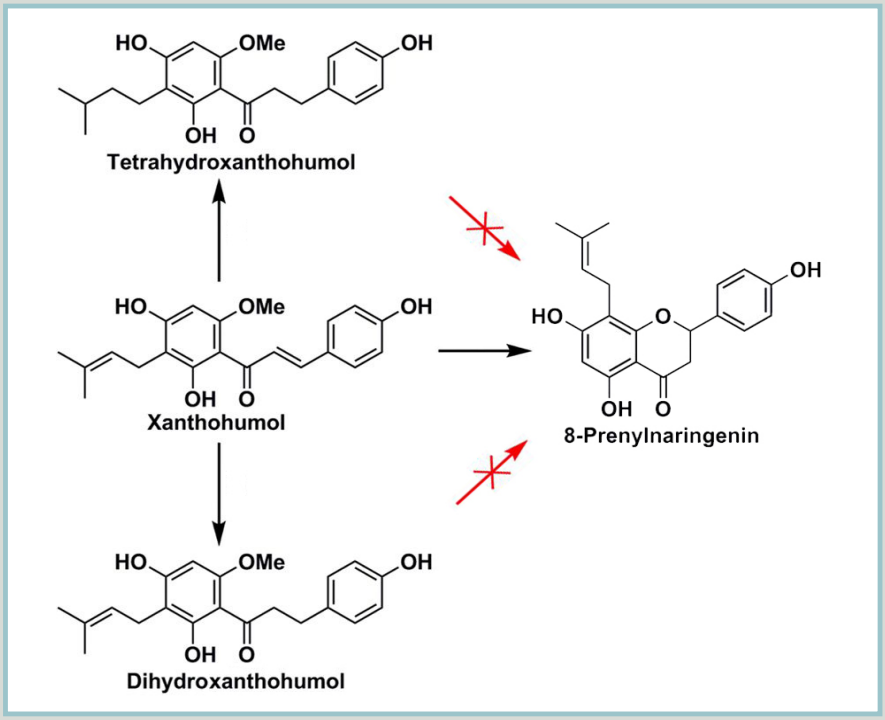
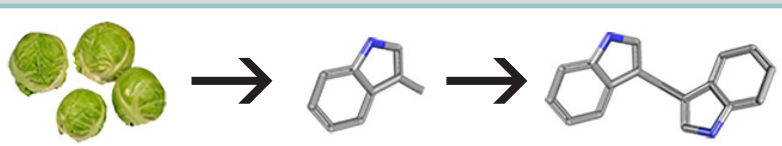


Dihydroxanthohumol and tetrahydroxanthohumol, the two reduced derivatives created by the Stevens laboratory, appear to share many of the same properties as xanthohumol with one key difference: they cannot be converted into the estrogenic metabolite 8-prenylnaringenin.

CANCER'S DIM OUTLOOK:

CAN EATING BRUSSELS SPROUTS BLOCK CARCINOGENS?



Glucobrassicin + Myrosinase → Indole-3-Carbinol (I3C) → Diindolylmethane (DIM)

The reaction of glucobrassicin and myrosinase from cruciferous vegetables releases indole-3-carbinol. Two molecules of indole-3-carbinol join together to produce DIM in an acidic environment, such as the stomach.

Diindolylmethane (DIM), a compound derived from phytochemicals found naturally in cruciferous vegetables, can stimulate multiple detoxification pathways in cells. Novel clinical studies by LPI investigator Dr. David Williams seek to determine how DIM can stop carcinogen exposure from leading to cancer.

Not everyone likes eating Brussels sprouts, including Dr. David Williams, the LPI Helen P. Rumbel Professor for Cancer Prevention. "I talk the talk," Dr. Williams admits, "But it is hard for me to walk the walk, even with everything I know about the cancer-fighting properties of cruciferous vegetables."

Members of the cruciferous vegetable family can have a strong flavor and odor; some people say they also have a bitter taste.

Yet there is some evidence that cruciferous vegetable intake is associated with a lower risk of certain types of cancers. Specific phytochemicals found in cruciferous vegetables may be responsible for this protection, leading many investigators to determine the exact mechanisms for this effect.

Various research groups at the Linus Pauling Institute study Phytochemicals. Drs. Laura Beaver and Emily Ho highlighted the cancer-fighting properties of sulforaphane from broccoli sprouts in an article in the Fall/Winter 2017 Research Newsletter.

Dr. Williams, on the other hand, focuses on Brussels sprouts and the phytochemical they can produce called diindolylmethane (often called DIM). While many cruciferous vegetables are considered a source of DIM, they do not produce DIM directly. Instead, DIM starts out as a compound called glucobrassicin in plant cells.

To form DIM, glucobrassicin must first react with an enzyme that is also present in the plant called myrosinase. This usually happens when the plant is damaged in some way. Crushing, chopping, blending, or chewing will work just as well to release the enzyme and start the reaction.

Once this happens, a compound called indole-3-carbinol is released and DIM can be produced **(see Figure on Page 8)**.

DIM and indole-3-carbinol have been the subjects of investigation for many cancer studies. One important finding is that these indoles seem to stimulate cellular detoxification processes. It is thought that if detoxification systems are stimulated in advance of exposure to a carcinogen, cancer protection results.

The Williams laboratory focuses on preventing these exposures to carcinogens. Their early studies focused on exposures to fish, specifically trout. More recently, they used mice to demonstrate that dietary indoles (like DIM) in the maternal diet could protect offspring from developing cancer later in life.

Their hypothesis is as follows: Eating Brussels sprouts will protect from carcinogen exposures, as the DIM would prevent at least some of the cellular damage these toxins can cause. Put simply, DIM may be a carcinogen blocker. Now they are conducting the clinical studies to support this concept.

In order to do this, Dr. Williams first had to tackle a few logistics – feeding Brussels sprouts to people was the easy part. To see if the provided DIM stimulates detoxification of carcinogens, they needed to find a way to expose people to that toxin, track its fate in the body, and still make it safe.

Dr. Williams has decades of experience studying polycyclic aromatic hydrocarbons (also called PAHs). Products of burning organic material (e.g., coal, gasoline, or wood), PAHs are found in the environment in varying amounts but also in essentially all food we eat to some degree. They are present at highest levels in smoked and charcoal grilled foods, due to the proximity to burning materials. The most studied of these PAHs called benzo[a]pyrene (or BaP) is extremely common, but a known human carcinogen.

Although we are exposed to PAHs every day, it is not in a controlled way. From experience, Dr. Williams knew that the small doses of BaP they planned to use in their study (about 45 nanograms – the weight of a grain of pollen) were well below the estimated amount one gets from dietary sources every day. An adult living in the US can be exposed to 270-750 ng per day.

By comparison, the amount of carcinogen consumed by participants in this study was far below a person eating some wood smoked salmon or a charcoal grilled hamburger. To track these extremely small amounts of carcinogen, there is only one solution: Dr. Williams needed to use radioactive isotopes.

While this might sound alarming, it is in actuality quite innocuous. Small amounts of radioactivity are found everywhere in our daily lives. The food that we eat and the water we drink are full of radioactive isotopes of carbon and potassium that are abundant on our planet.

> "Imagine the ability of detecting the equivalent of a single drop of blood in a volume the size of a small lake."
>
> —David Williams, PhD

"To put this in perspective," Williams explains, "The amount of radioactivity we are using is the equivalent to that found in five bananas and less than one percent of a dose that is given to patients to diagnose *Helicobacter pylori* infections."

The radiation dose is measured at five nanocuries, roughly equivalent to a routine X-ray. However, to make sure that this exposure was safe to all participants, the National Institutes of Health, the Food and Drug Administration, and Oregon State University's Institutional Review Board all reviewed and gave Dr. Williams permission to proceed with the study.

The advantages in using radioactive isotopes are quite clear to researchers: The labeling allows researchers to use very small quantities and still find them within the body. Radioisotopes provide extra mass to these molecules, allowing them to distinguish isotope-labeled products from unlabeled products – at least, with a little help from a particle accelerator.

Collaborators at Lawrence Livermore National Laboratory's Center for Accelerator Mass Spectrometry have been working with the Williams lab for years to analyze plasma and urine samples from human volunteers. With their accelerator mass spectrometer technology (AMS), the sensitivity of measurements has dramatically increased. "AMS combined with radioisotopes gave us astounding levels of sensitivity," says Dr. Williams, "Imagine the ability of detecting the equivalent of a single drop of blood in a volume the size of a small lake."

In order to see the effects of DIM, each volunteer needs to consume the radiolabeled BaP alone and in a separate trial with the inclusion of Brussels sprouts or the DIM capsules. Metabolic products from the BaP are quantified, including any that associate with DNA, and the differences between the two trials will show exactly the effects of DIM – and the whole food from which the metabolites are derived – on carcinogen metabolism.

As Dr. Williams notes, "This is the first human trial to determine if a cruciferous vegetable, or a food-derived dietary supplement, can alter the risk from ingesting a known carcinogen at an environmentally relevant dose. To date, we have had to rely on risk assessment from data from animals dosed with 10,000 to 1 million times the exposures people face on a daily basis."

This trial is currently underway and the details are found at **clinicaltrials.gov** under study number NCT03631667. We will have more insights to report from the study as it proceeds.

The health benefits of cruciferous vegetables likely stretch beyond DIM and sulforaphane. So even if you are not a big fan of Brussels sprouts in particular, broccoli, cabbage, or kale can be a healthful addition to your diet.

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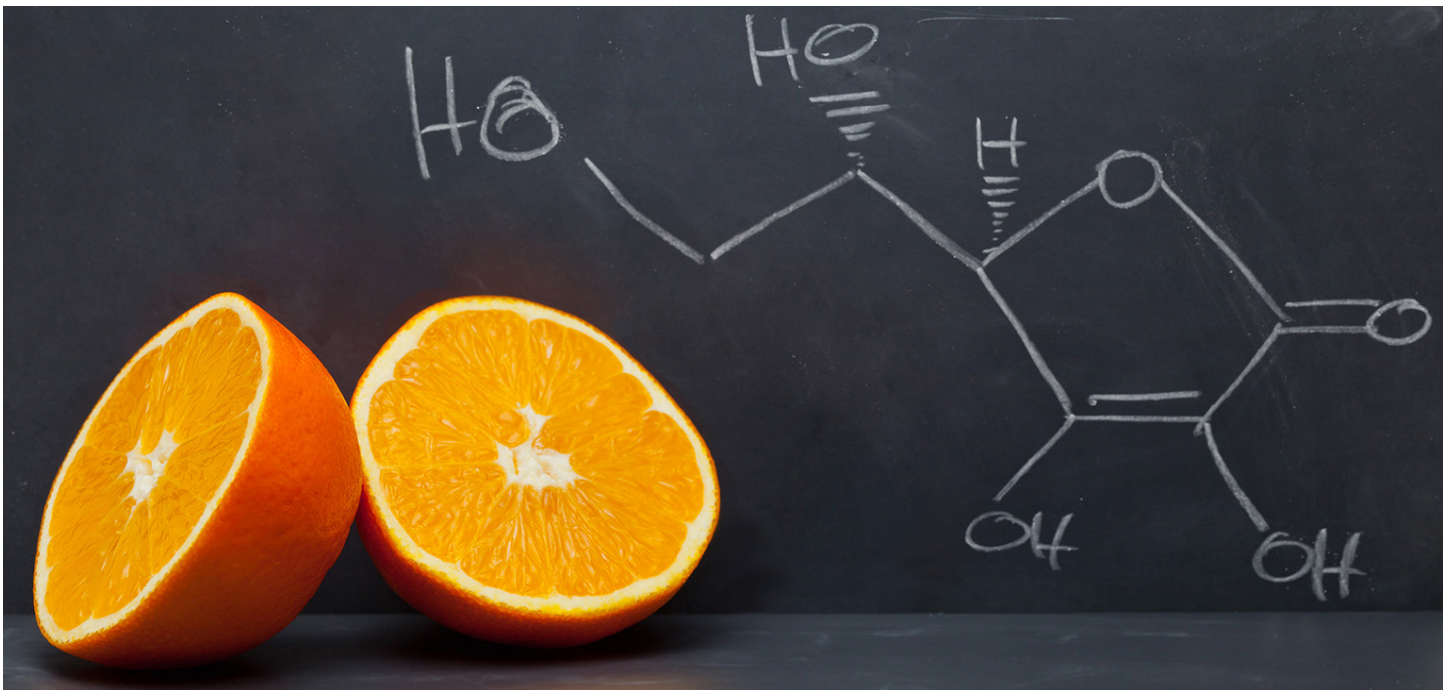

FREQUENTLY ASKED QUESTIONS ABOUT VITAMIN C

As you might imagine, we field many questions about Linus Pauling's favorite molecule: vitamin C. Below, we highlight some of these questions focusing on supplemental forms of vitamin C. If you have any additional questions that are not covered here, or have topic suggestions for our next FAQ story, please email us at lpi@oregonstate.edu.

What brands of vitamin C should I take?

The Linus Pauling Institute does not recommend any particular brand of dietary supplements. However, we do have some general advice when choosing supplements. Look for brands that bear the "USP verified" or the "NSF certified" seal; these products pass verification standards for quality, purity, and potency. Additionally, there are online resources like Consumer Reports, consumerlab.com and labdoor.com that list results of quality testing.

In general, you do not need to take expensive vitamin C supplements. A supplement that contains ascorbic acid is sufficient to meet your needs. See the next questions for information about different types of vitamin C formulations.

Should I take "buffered" vitamin C?

Many people choose to take mineral forms (often called 'buffered' due to its neutral pH) of vitamin C if the acidic form (ascorbic acid) upsets their stomach. If taking vitamin C gives you heartburn, try a mineral ascorbate – these come in calcium, magnesium, or sodium ascorbate salts.

Be aware: sodium ascorbate will increase your total intake of sodium and should probably be avoided in high amounts. In addition, there are some data to suggest that calcium ascorbate is not absorbed as well as other forms of ascorbate.

Does the Institute recommend liposomal vitamin C?

Despite all the questions about liposomal vitamin C (and we get a lot of them) and the testimonials we have heard, there is little research on this form of vitamin C. Once carefully controlled scientific studies are published, we will have much more to say on the topic.

Is it true that natural vitamin C is better than synthetic vitamin C because it exists in a complex of factors?

Absolutely not. There is no difference between natural and synthetic vitamin C. This is a very common myth so it is difficult to trace the source, but we believe that it came from early studies by Albert Szent-Györgyi, the man who is credited with the discovery of vitamin C's molecular structure.

At the time Szent-Györgyi accepted the Nobel Prize, he thought he had evidence suggesting that vitamin C needed other molecules, such as flavonoids, found in the same plants in order to function properly. He hypothesized they formed complexes together that aided absorption. However, Szent-Györgyi's theory did not pan out, and he admitted to this later in his career.

Ascorbic acid is vitamin C because it alone can prevent and cure scurvy, making it an essential micronutrient – the basis its classification as a vitamin.

Is it important to take ascorbic acid with flavonoids?

There is no evidence to suggest that taking vitamin C with flavonoids is particularly helpful. On the contrary, one study that investigated vitamin C absorption with flavonoids showed that quercetin (one flavonoid found in rose hips and onions)

could potentially block some vitamin C absorption. The magnitude of this effect is unclear – the amount of vitamin C blocked from absorption may be trivial. However, it certainly does not suggest any particular benefits to combining flavonoids with vitamin C.

Why do some people take vitamin C intravenously?

The body has proteins in the small intestine dedicated to vitamin C absorption. These proteins are saturated by taking a relatively small amount of supplemental vitamin C. Studies have shown that dosages of 500 mg or higher are not fully absorbed in healthy young individuals. This effectively limits how much vitamin C can enter the bloodstream.

To bypass this absorption system, intravenous vitamin C is used to achieve blood concentrations of ascorbic acid up to 1000-times higher than what can be achieved by taking dietary supplements. These concentrations are thought to be beneficial in the immune system and have been used in cancer therapies (see next question), but we really do not know what high-dose vitamin C can do in the body.

Currently, intravenous vitamin C is being used in a drug cocktail to successfully treat sepsis. For more information on this, look for articles on Paul Marik's work at Eastern Virginia Medical School, and see the latest update of the vitamin C article on the Micronutrient Information Center website (coming soon).

Although extremely high doses of vitamin C given by IV have few known side effects, they should only be administered in consultation with a medical professional.

Can intravenous vitamin C be used as a cancer therapy?

Despite Linus Pauling's work with Ewan Cameron using intravenous vitamin C in cancer patients over 40 years ago, it is still considered an unproven cancer therapy. Although there are several new studies about the cancer-fighting properties of intravenous high-dose vitamin C, the treatment is not always effective. At the moment, physicians providing intravenous vitamin C usually do so in combination with standard therapies (such as chemotherapy), and not as a sole method of treatment. In order for intravenous vitamin C to move into the mainstream, we need more and better research to learn what can make it more effective.

Some caution is warranted when considering intravenous vitamin C: Several types of cancer cells appear to be resistant to its effects. Indeed, the mechanisms behind vitamin C's cancer-fighting activity still elude us.

High amounts of vitamin C can interfere with certain types of chemotherapy and should never be taken in combination with other drugs or therapies without consultation with a physician. Be aware of these facts when exploring your treatment options.

What are your recommendations for people taking the "Pauling Therapy" to treat heart disease?

The **Pauling Therapy** is a nickname given to the combination of vitamin C, lysine, and proline that was recommended by Linus Pauling to fight atherosclerosis. He believed that these nutrients, when taken in large doses, would slow deposition of oxidized lipoproteins in the arterial wall, preventing heart attacks or other serious cardiovascular conditions. This combination was also intended to bolster the collagen in blood vessels, making them stronger and more resilient.

Unfortunately, there is not a good foundation of scientific or medical research behind the use of this combination of supplements to fight heart disease. Much of the clinical work needed to support this therapy still needs to be done.

Despite this, many people around the world testify to the power of supplemental vitamin C with or without lysine and proline added. We appreciate the experiences you have shared with us over the years.

In short, until the science evaluates this combination in earnest, the Linus Pauling Institute will not make any official comment on the use of this therapy.

Do large amounts of vitamin C cause any harmful effects?

They might. People who take large amounts of vitamin C often report gastrointestinal side effects. This can range from gas and bloating to diarrhea. This is because large doses are not completely absorbed. The amount that can cause these issues varies from person to person, but the Tolerable Upper Intake Level is 2000 mg per day for adults to help minimize the risk of these effects.

Since ascorbic acid can break down to form oxalate, individuals who produce oxalate kidney stones might be at risk for developing stones more frequently. A few studies have reported an increased risk for kidney stone formation in people who have taken vitamin C supplements, but some studies have found no increased risk. Given that there is a possibility, we recommend that people who are prone to kidney stones avoid taking high doses of supplemental vitamin C.

Why do we still know so little about vitamin C?

Vitamin C is not considered a priority for government funding. There is a general perception that there is not more to learn about vitamin C after many decades of research.

However, we believe that there is much more to learn about this amazing molecule. The state-of-the-art technologies at the Linus Pauling Institute can help us move forward in this regard.

If you would like to help support future studies on vitamin C, please contact us.

What if I have more questions about vitamin C?

The LPI's Micronutrient Information Center has an excellent page on vitamin C that is reviewed by experts in the field: **lpi.oregonstate.edu/mic/vitamins/vitamin-C**

Lastly, you can always email or call us with any additional questions you may have.

BIOACTIVES, BOTANICALS, AND REDOX MECHANISMS

LINUS PAULING INSTITUTE 10th INTERNATIONAL CONFERENCE AND SFRBM REGIONAL SYMPOSIUM

AUG 14-16, 2019

OREGON STATE UNIVERSITY - CORVALLIS, OREGON

The LPI's 10th biennial conference is presented in conjunction with the Society for Redox Biology and Medicine. Join us for this three-day event highlighting the emerging roles for botanicals and other bioactives, the role of redox signaling in disease processes, and how they come together for therapeutic benefit.

CONFERENCE SESSIONS

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- Tumor Biology
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- Mechanisms of Action for Botanicals and Other Bioactives
- The Regulation and Standardization of Botanicals
- New Approaches to Botanicals

CONFERENCE CHAIRS

- **Richard van Breemen, PhD,** *Oregon State University*
- **Maria Clara Franco, PhD,** *Oregon State University*

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MORE INFORMATION

lpi.oregonstate.edu/conference

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LPI STUDENTS

Daniel Nosal

PhD Student, College of Pharmacy

Born in Chicago, Daniel Nosal spent his childhood in the suburban area. His drive toward a science career began when he was involved in his father's construction company: Observing the manipulation of raw materials to form useful products fueled a desire for creation through chemistry. This propelled him to study organic chemistry at the University of Illinois at Chicago.

The interactions between small molecules and proteins intrigued Daniel during his undergraduate years. He soon joined a laboratory focused on developing new pharmaceutical drugs. Here he learned about synthetic chemistry, instrumental analysis, and the use of incredibly sensitive equipment to make discoveries about the chemical world.

Daniel's pursuit of a cutting-edge approach to solve fundamental questions about chemistry and biology led him to join the lab of Dr. Richard van Breemen for his graduate work. Here, Daniel analyzes natural products for their bioactive components. The methodologies Daniel is currently developing in the van Breemen laboratory are designed to fuel the discovery of novel drugs and improve human health.

When the Linus Pauling Institute asked Dr. van Breemen to be its new director, Daniel did not hesitate to follow him to Oregon State University. With the knowledge and skills he gains in the van Breemen laboratory, Daniel seeks to develop the next generation of scientific tools to discover new bioactive compounds from botanical sources that have the potential for saving lives.

Luying Chen

PhD Student, College of Pharmacy

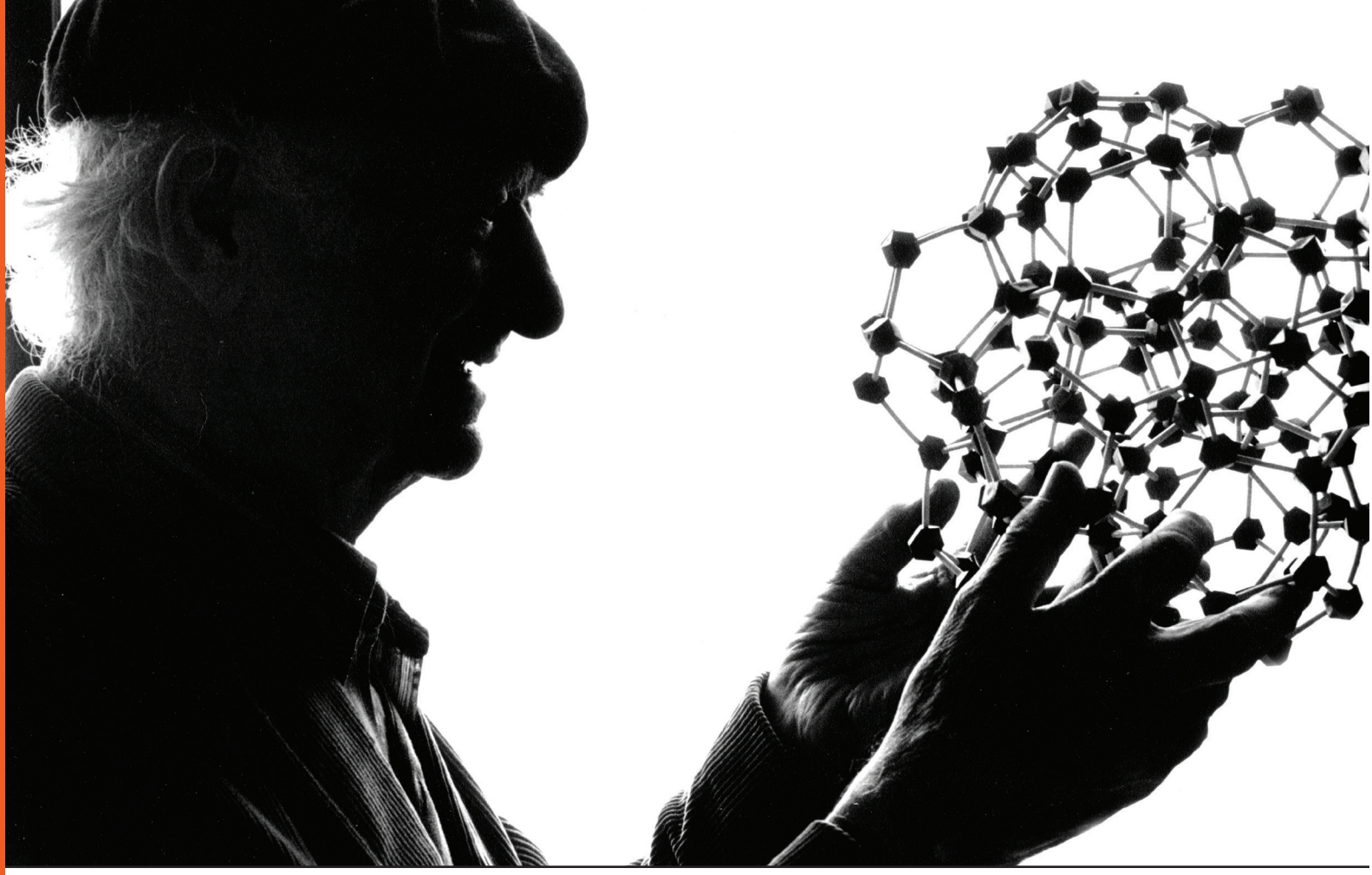
Luying Chen was born and raised in Harbin, a multicultural city in Northeastern China. She traces her interests in human health and science back to early childhood, as her mother is a doctor and her father is a university chemistry professor.

After receiving an undergraduate degree from Sun Yat-sen University, Luying attended Shanghai Institute of Materia Medica in the Chinese Academy of Sciences, and achieved her Master's degree in medicinal chemistry. With a focus on natural product chemistry and drug metabolism, her studies provided an appreciation for traditional Chinese medicines.

In China, disease treatment often involves a combination of traditional medicines and 'Western medicine.' Luying's rigorous education focused on the metabolism of active ingredients found in the plants used for traditional Chinese medicine. This included characterizing the metabolites that are developed after administering these compounds to animals.

Now a student working with Dr. Richard van Breemen, Luying has learned more applications in mass spectrometry, which she calls "a technique that combines tradition with innovation." During two internships in the pharmaceutical industry, she explored the use of these technologies in drug discovery.

Her dissertation work focuses on determining the interactions between botanical dietary supplements and drugs, a common issue for people around the globe. After graduation, she plans to develop new mass spectrometry bioassays for use in drug development.



Carry the Legacy Forward

MORE THAN 40 YEARS AGO, Linus Pauling concluded that vitamins and other essential micronutrients play a significant role in enhancing health and preventing disease. He entrusted his alma mater, Oregon State University, with continuing his work and his legacy through the Linus Pauling Institute.

Through a planned gift that benefits LPI, you too can create a legacy. It's as easy as including the OSU Foundation in your will or as a beneficiary of your retirement plan. You have many options. **Contact us to learn more.**

Julie Irmer, Office of Gift Planning
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*Today, **Richard van Breemen** leads the institute that Linus Pauling founded to promote optimal health.*

Oregon State University Foundation

OUTREACH

The **Healthy Youth Program** promotes optimal health for youth and families through hands-on education. Since economic and societal factors can influence health in communities, their team has stepped up efforts to provide programming to traditionally underserved families.

Through support from the Linn Benton Health Equity Alliance, a new Healthy Youth Program's Bilingual Education Assistant, Sandra Mecinas-Estevez, was hired in April 2018. An OSU student with a declared major in Human Development and Family Sciences and pursuing a second major in Elementary Education, Sandra is translating the materials used in the family cooking classes and elementary school garden-based activities into Spanish.

Sandra will collaborate with Julie Jacobs, Healthy Youth Program Nutrition Educator, to offer a family cooking class in Spanish in Lebanon, Oregon through the Culinary Health Education and Fitness (C.H.E.F.) Program. At Lincoln Elementary School in Corvallis, the school garden-based curricula will now be available to all English- and Spanish-speaking classrooms.

The Healthy Youth Program has committed to provide scholarships for up to 30% of registrants in their fee-based cooking classes for youth. With the help of Community Health Navigators through the Benton County Health Department, low-income families are directed to these opportunities. As a result of this partnership, target enrollment has been reached for nearly all of Healthy Youth Program's youth classes in Corvallis this year.

HOW THE MICRONUTRIENT INFORMATION CENTER IS EXPANDING ITS REACH AND UTILITY

In collaboration with faculty members at Niigata University of Pharmacy and Applied Life Sciences (NUPALS), the Japanese version of the popular **Micronutrient Information Center** was recently launched. This can be found on our website at ***lpi.oregonstate.edu/jp/mic***.

Select articles on micronutrients and phytochemicals are currently available on the Japanese site, and additional translations will be posted in the future.

Now offered in three different languages (the Spanish Micronutrient Information Center debuted in 2013), the freely available, evidence-based content on micronutrients is accessible to more of the global population.

The Micronutrient Information Center will also launch the LPI's first online, continuing education courses; these courses are specifically designed for healthcare professionals but open to anyone interested in the subject matter. One course will cover micronutrient inadequacies in the United States population, including subpopulations at risk. Another course will explore the roles of nutrients in achieving and maintaining bone health throughout the lifespan.

Acknowledgments go to Pfizer, Inc. for awarding the grants that allowed the development of these courses, as well as the curriculum designer at Oregon State University's Professional and Continuing Education who developed the online course material.

lpi.oregonstate.edu/mic

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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For over 45 years, the Linus Pauling Institute has work to enhance health and fight disease. Today, you have an opportunity to establish your own legacy in health science by supporting the LPI. Contact the OSU Foundation at osufoundation.org/GiveToLPI or call (800) 354-7281

Introducing Dr. Melissa Haendel and her team in the Translational and Integrative Sciences lab. LOOK INSIDE ►