**SULFORAPHANE AND THE MICROBIOME**

Sulforaphane, a compound with roles in cancer prevention, does not naturally occur in plants. Instead, cruciferous vegetables contain glucoraphanin that can be converted to sulforaphane. Recent research from Dr. Emily Ho’s group shows another possible fate for glucoraphanin: conversion to sulforaphane nitrile. Their data support new relationships between sulforaphane metabolism, gut bacteria, and personalized nutrition.

Glucoraphanin found in broccoli, cauliflower, kale, cabbage, mustard greens, and Brussels sprouts can be converted to sulforaphane through a reaction catalyzed by an enzyme called myrosinase, which is also found in these vegetables. When these plants are crushed, chopped, or chewed, the enzyme reacts with glucoraphanin to produce sulforaphane (for more details, see “Broccoli FAQ” in the March-May 2022 LPI Research Newsletter).

Broccoli is a good source of glucoraphanin. Broccoli sprouts (three to four-day-old broccoli plants) are an excellent source of glucoraphanin. By weight, broccoli sprouts contain 10-100 times more glucoraphanin than a mature plant, making them a convenient way to maximize the health benefits of sulforaphane.

Broccoli sprouts are often used in clinical trials to study sulforaphane. Dr. Emily Ho has used broccoli sprouts in clinical trials for cancer prevention. These sprouts are inexpensive, easy to grow, and a readily available source of glucoraphanin and myrosinase.

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FROM THE DIRECTOR

I hope everyone is having a wonderful 2024! The first half of this year has been a great time for the Institute.

First, we had a very successful Linus Pauling Day event in February that started with a webinar on the Mediterranean diet. It continued with an open house in Corvallis to showcase our research. We had a fabulous time! It was amazing to see many of our LPI supporters engaging with our researchers and students and to have so much incredible health science on display.

On April 2, I served as a moderator for author Michael Pollan’s presentation for the Oregon State University Provost’s Lecture Series. It was a great honor to accompany him throughout the day, talk with him about his books, and introduce him to students. It was a packed crowd at OSU’s Austin Auditorium for his presentation, which was one of the most-watched lectures at OSU.

At the end of April, we geared up for Dam Proud Day, OSU’s annual day of giving. This year we focused on support for our Healthy Aging Research Fund and the Micronutrient Information Center.

By all measures, this year’s Dam Proud Day efforts were an enormous success. We raised more than $90,000 for our outreach and research programs and received gifts from more people than ever before. The Institute was ranked as the 10th (out of 53) highest fundraising unit on campus. We are sincerely grateful for your commitment to health research.

We also recently unveiled a new resource highlighting 10 vitamins and minerals many people need to consume more, including several helpful nutrients for people over 50 years of age. The first phase of this project includes a detailed informational brochure and several accompanying materials.

The preview of this project can be seen in our latest webinar Aging Well: The Top 10 Micronutrients for Optimal Health, which reviews this information with a Q&A session from our nutrition experts. Details about the brochure and some questions answered by our experts will be available in the next issue of the LPI Digital Digest.

With the end of the academic year, our students can devote more time to their research at the Institute. Thanks to generous donations, we have introduced a new LPI Summer Research Scholars Program that will support research projects for seven students this year (see next page). We will share some highlights of their research with you soon.

Coming soon is another installment of our LPI webinar series. This webinar will be presented by Dr. Richard Bruno, an LPI alumnus who previously worked with Dr. Maret Traber. Although the date and time are yet to be finalized, it will occur in late August and will focus on some of the health benefits of green tea extracts. The time and date of this webinar will be posted on our website and sent by email.

I wish everyone a healthy and productive summer! Another research update will be coming your way in the fall.

All our webinars can be found on our YouTube channel.

Living Better, Longer: Healthy Compounds Found in a Mediterranean Diet
lpi.pub/MedDiet2024

Aging Well: The Top 10 Micronutrients for Optimal Health
lpi.pub/Top10Webinar

Two issues of the Institute’s Digital Digest have been published. Did you get yours?

The Digital Digest is primarily published online, but if you request it from us (use the enclosed envelope or give us a call), we’ll send you a copy in the mail at no charge!
A NEW RESEARCH PROGRAM FOR STUDENTS

This year, a generous gift to the Linus Pauling Institute made possible the launch of the LPI Summer Research Scholars Program. This program is an opportunity for students to work with a faculty mentor within the Institute and gain research experience in areas that support the Institute’s mission to promote optimal health through cutting-edge research.

In the inaugural year of this program, seven OSU students were selected to participate based on their scholarly achievements and the quality of their scientific proposal, as evaluated by LPI faculty. Each student will present the results of their summer research in a public presentation.

CARMEN BROWN
Department: Biochemistry and Molecular Biology
Faculty Advisor: Dr. Alysia Vrailas-Mortimer
Project Title: Age’s Effect on Neurodegenerative Diseases

LAUREN CLAYTON
Department: Biology
Faculty Advisor: Dr. Alysia Vrailas-Mortimer
Project Title: Testing Drugs on a Fly Model of Parkinson’s Disease

ARIELLE DAMANIA
Department: Biology
Faculty Advisor: Dr. Alysia Vrailas-Mortimer
Project Title: The Effects of GARS-CMT Disease on Muscular Degeneration in Drosophila

HAILEY HARRIS
Department: Psychology
Faculty Advisor: Dr. Alysia Vrailas-Mortimer
Project Title: Empagliflozin Influence on Lifespan and Locomotive Function in Drosophila melanogaster

PAIGE JAMIESON
Department: Nutrition
Faculty Advisor: Dr. Fred Stevens
Project Title: Impacts on Microbial Metabolism in Response to Xanthohumol Supplementation in Crohn’s Disease Utilizing an In Vitro Simulation Model

NATALIE THUN
Department: BioHealth Sciences
Faculty Advisor: Dr. Richard van Breemen
Project Title: Drug-Botanical Pharmacokinetic Interactions: HIV Drugs and Kratom
In previous clinical trials, researchers in the Ho laboratory found that blood levels of sulforaphane varied from person to person despite participants consuming the same amount of glucoraphanin. Ho’s research group designed a clinical trial to investigate these differences.

Recently published in the journal *Molecular Nutrition and Food Research*, the study showed some unexpected findings relating to sulforaphane metabolism.

**Following Sulforaphane Production**

The clinical trial was conducted in 55 healthy men and women between 19 and 60 years old. Each participant was provided a standardized quantity of broccoli sprouts or an equal amount of alfalfa sprouts as the control treatment.

After sprout consumption, the researchers collected blood, urine, and stool samples for 72 hours. Then the researchers measured sulforaphane and its metabolites in all of the samples to create a cohesive picture of sulforaphane metabolism.

Samples from participants consuming alfalfa sprouts did not contain sulforaphane or its metabolites. Alfalfa is not a cruciferous vegetable and does not contain glucoraphanin, so these results were expected.

In individuals consuming broccoli sprouts, the stool samples provided the first indication of what was happening to the glucoraphanin as it was released from the sprouts and passed through the digestive tract. These samples contained a notable amount of free sulforaphane – especially those collected within the first 24 hours.

A few sulforaphane metabolites were present in the stool samples, but they were only abundant in samples from a few individuals.

By contrast, the blood and urine samples contained very little free sulforaphane and much higher concentrations of sulforaphane metabolites. This was expected as sulforaphane is rapidly metabolized and eliminated from the body.

A compound known as sulforaphane nitrile was the most abundant metabolite found in many of the blood samples. Although sulforaphane nitrile has been found in previous studies on cruciferous vegetables, the amount of the nitrile compound found in these blood samples was surprising.

**Finding the Source of Sulforaphane Nitrile**

So, what is sulforaphane nitrile? There are some basic similarities in the chemical structures of sulforaphane and sulforaphane nitrile (see figure below), as both molecules are derived from glucoraphanin.

However, there are some substantial differences in the chemical properties of the two compounds. Sulforaphane is a very reactive molecule, while the nitrile form is relatively stable.

Tests in cell culture performed by Ho’s group confirmed that sulforaphane nitrile does not exhibit anticancer properties, even at relatively high concentrations. This means that there are likely no health benefits to sulforaphane nitrile.

To learn how to grow your own broccoli sprouts, see Dr. Ho’s webinar, *Cancer Fighter: Harnessing the Power of Broccoli* on YouTube at [lpi.pub/BrocVsCancer](http://lpi.pub/BrocVsCancer)
An interesting finding was that blood samples from some individuals contained much more sulforaphane nitrile than samples from other participants. The most likely explanation for this individuality in the data is differences in gut bacteria, as previous data showed that only some types of gut bacteria are able to metabolize glucoraphanin into sulforaphane nitrile.

Since each person’s gut microbiome contains different types and amounts of bacteria, these differences could explain the individual variation in sulforaphane nitrile levels.

When the research team analyzed the types of bacteria found in the stool samples, they found a few candidate bacterial species that might have produced sulforaphane nitrile in the digestive tract. There was also a correlation between the amounts of these bacteria and the levels of sulforaphane nitrile in the blood samples.

Although this is not direct evidence that the gut bacteria created sulforaphane nitrile, it is plausible to draw this conclusion. Previous work in the Ho laboratory revealed that human fecal cultures are capable of producing sulforaphane nitrile.

Since relatively high amounts of sulforaphane nitrile were found in the blood samples, sulforaphane nitrile is likely well absorbed after being produced in the gut. The presence in the urine samples for up to 72 hours suggests this compound is also being excreted but relatively slowly.

**Optimizing Sulforaphane Production**

The production of sulforaphane nitrile is not the desired outcome after eating cruciferous vegetables. Although sulforaphane nitrile does not appear to be harmful, it could have some effects that are not yet known. However, if you are a person who generates more of this nitrile compound, you are likely making less sulforaphane since both reactions are dependent on the amount of glucoraphanin you consume.

Therefore, you may be receiving less of the health benefits of eating broccoli.

A simple strategy to combat this effect is to optimize the conversion of glucoraphanin to sulforaphane before it reaches the intestine. One way to do that is to avoid cooking your cruciferous vegetables to the point they become limp. Raw or lightly cooked vegetables retain myrosinase, the enzyme that converts glucoraphanin to sulforaphane.

The focus of future research, however, is on the gut microbiome. Although this study indicates that some gut bacteria may be responsible for sulforaphane nitrile production, other studies show that some gut bacteria foster sulforaphane production in the gut without needing myrosinase. Thus, researchers are trying to determine how changes in diet and the gut microbiome can maximize sulforaphane production while minimizing sulforaphane nitrile generation.

Overall, this research study on sulforaphane metabolism highlights the need to better understand inter-individual differences to inform personalized nutrition.

**References**


Overcooking broccoli can destroy myrosinase, the enzyme needed to produce sulforaphane. However, ground mustard powder is a good source of myrosinase. Use it to season cooked broccoli or add to sauces or dressings in your meal.
Oxylipins are a collection of molecules that are formed when polyunsaturated fatty acids (also known as PUFAs) undergo oxidation. Detecting oxylipins in biological fluids or oils can be tricky, but a new method developed by investigators at the Linus Pauling Institute helps make it faster and easier than before.

Nuts, seeds, most vegetable oils, and oily fish are good sources of polyunsaturated fatty acids (also known as PUFAs), and a diet rich in PUFAs is associated with many health benefits.

Compared to saturated fats, however, unsaturated fats like PUFAs are much more prone to oxidation, an unavoidable and undesirable outcome. Our bodies create reactive oxygen species as a by-product of normal metabolism. Oxidation occurs as a by-product of normal metabolism, but additional oxidation can occur when cells are stressed.

When oxidized, PUFA molecules can undergo chemical changes. These oxidized molecules may join together, break apart, or change shape. Scientists refer to the resulting new molecules as “oxylipins.”

Oxylipins are not necessarily good or bad – some have biological properties, and some do not – but all indicate that oxidation has occurred.

Detecting oxylipins can be tricky for two reasons. First, they are very low in abundance. There may be only one oxylipin molecule per tens of thousands of unoxidized PUFA molecules. Second, oxylipin is an umbrella term encompassing over one hundred different molecules with an array of chemical structures and properties.

It is clear that finding and quantifying oxylipins requires sensitive and specific techniques. Drs. Claudia Maier and Fred Stevens, investigators within the Linus Pauling Institute and directors of the Mass Spectrometry Center at Oregon State University, led a research team to develop a new method to detect oxylipins in biological samples, such as blood samples.

Their results were recently published in the journal Current Protocols. This new technique can simultaneously quantify up to 88 different types of oxylipins in samples, more than previously reported. It is also faster than previous methods and takes full advantage of the sensitivity afforded by mass spectrometry analytical techniques.

Not only does this allow for quick and accurate monitoring of fatty acid oxidation, but the protocol is adaptable for multiple applications.

For example, the presence of oxylipins in a blood sample might indicate the presence of inflammation or a disease. Previous research at the Linus Pauling Institute has linked the presence of oxylipins with the development of coronary artery disease.

This method could also be used to monitor the oxidation of cooking oils or other edible oils. Another potential application is to monitor oxidation in fish oil supplements – a perennial issue for the dietary supplement industry.

This new analytical method will enable deeper and more efficient investigations of the negative consequences of PUFA oxidation within our bodies, supplements, and foods.

References
Each vitamin has its unique properties that make it essential for life. Vitamin E was first discovered when researchers found that laboratory rats were unable to reproduce. An essential factor was missing in the diet and was needed for the early stages of embryonic development. Without it, the embryos did not survive.

Careful experimentation in the 1920s revealed that the missing factor was present in wheat germ. Scientists then turned their efforts to isolating this molecule. Thus came the discovery of alpha-tocopherol, a lipid-soluble compound often found in the plasma membranes of cells.

However, this wasn’t the end of the story. Not too long after the discovery of alpha-tocopherol came the identification of three related molecules: beta-, delta-, and gamma-tocopherol. Some years later, a similar group of molecules called the tocotrienols was also found in plants.

Together, the tocopherols and tocotrienols are correctly classified as “tocochromanols” but are more commonly referred to as “eight forms of vitamin E.” As Dr. Maret Traber discussed in a recently published review in the journal Free Radical Biology and Medicine, only one molecule deserves vitamin status: alpha-tocopherol, the specific molecule that prevented the death of developing embryos.

In her review, Traber outlined the consequences of vitamin E deficiency in humans, which typically manifests as neurological abnormalities in people with prolonged, severe dietary deficiencies. Only alpha-tocopherol can reverse or prevent the progression of these issues and restore health.

Traber also noted that our bodies have a specific alpha-tocopherol transfer protein, which only recognizes alpha-tocopherol. A mutation in the gene for the alpha-tocopherol transfer protein results in a condition known as Ataxia with isolated Vitamin E Deficiency or AVED.

Symptoms of AVED include numbness in the hands and feet, leg weakness, and ultimately an inability to walk – all symptoms that point to the essential role of vitamin E in nerve function.

Vitamin E deficiency symptoms are also associated with other disorders involving the absorption and movement of alpha-tocopherol in the body. For example, people with genetic disorders that affect the absorption or transport of fat can become vitamin E deficient. This is because alpha-tocopherol is transported similarly to fats.

Defining the molecular identity of vitamin E might seem unnecessary, but it is important for setting dietary intake recommendations. Alpha-tocopherol is the only molecule that will prevent vitamin E deficiency and therefore is considered essential. Other molecules cannot satisfy this role.

This is not to say that the other tocopherols and tocotrienols do not have biological activity. Like many phytochemicals, tocochromanols have a role to play in promoting health, but further research is needed to properly define their purpose.

References

Maret Traber, PhD
Principal Investigator
Linus Pauling Institute

In addition to genetic disorders, people who have had intestinal surgery or take medications that reduce fat absorption are at risk for vitamin E deficiency.

Vitamin E is abundant in many plants that contain fat, like avocados, nuts, seeds, and oils. It can also be found in green leafy vegetables.

Find out more about vitamin E at the Micronutrient Information Center at lpi.pub/VitE
Thank you for your generous support during Dam Proud Day!

Whether you donated, attended our Top 10 webinar, or spread the word about the Institute to your friends and family, your efforts played a pivotal role in our annual day of giving at the Linus Pauling Institute.

Because of you, we raised over $90,000! Your giving yields discoveries in healthy aging and nutrition and helps broadcast our message worldwide. Together, we are making strides toward a world where health and well-being flourish.

THANK YOU FOR BEING A VITAL PART OF THE LINUS PAULING INSTITUTE!