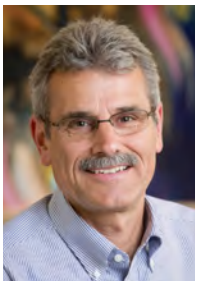
OSU Oregon State University

The Linus Pauling Institute

SPRING-SUMMER 2016 **RESEARCH NEWSLETTER**



From the Director

Balz Frei, Ph.D.

LPI Director and Endowed Chair
Distinguished Professor of Biochemistry and Biophysics
Joan H. Facey LPI Professor

As announced in my last newsletter column, I have stepped down as director and endowed chair of the Linus Pauling Institute and retired from Oregon State University. It has been an honor and privilege to serve the LPI and OSU for almost 20 years and help build the institute from its modest beginnings to where it is today in the new, state-of-the-art Linus Pauling Science Center. At my retirement celebration, I thanked the many people who critically contributed to the institute's success, including Barbara McVicar, my loyal assistant and personal advisor, who kept the institute running smoothly all those years; Stephen Lawson, who served the institute for almost 40 years as Dr. Linus Pauling's right-hand man and CEO of the Linus Pauling Institute of Science and Medicine in Palo Alto, CA, and administrative officer, newsletter editor, and institutional memory of LPI at OSU; and the university's president, Dr. Ed Ray, for his strong support and advocacy of LPI and having had the courage and vision to start OSU's first capital campaign, which provided the funding for the institute's fabulous new research facilities.

Barbara McVicar

Stephen Lawson

Ed Ray

I also thanked the institute's 12 principal investigators. When I arrived at OSU in 1997 from the Boston University School of Medicine, my simple "recipe" for a successful

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Skin Health

An Interview with
Arup Indra, Ph.D.
LPI Adjunct Faculty

Q. Were you born in India?

A. Yes.

Q. Where were you educated?

A. I did my schooling and attended college in India.

Q. Did you earn your doctorate in India as well?

A. I did. I earned my Master's degree in Biochemistry at the University of Calcutta, one of the oldest universities in India, and then earned my Ph.D. in Cell and Molecular Biology from Bose Institute, Kolkata, one of the premier research institutes in India.

Q. Where did you begin your career?

A. I moved to Europe for my postdoctoral training right after my doctorate in India and worked in the Institut Génétique Biologie Moléculaire Cellulaire (IGBMC) in Strasbourg, France, under Professor Pierre Chambon, who is called the guru of nuclear receptors and won the Albert Lasker Award for Basic Medical Research in 2004. IGBMC is regarded as one of the premier institutes in the world in molecular biology.

Q. What brought you to Oregon State University?

A. That's an interesting question. After close to 10 years of working as a postdoctoral fellow and as a research scientist, my mentor Pierre felt that I should be independent to progress in this area. That's when I found a very good opportunity here. Oregon State University seemed like the perfect place to grow and establish myself.

Q. What department are you in at OSU?

A. The Department of Pharmaceutical Sciences in the College of Pharmacy.

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Continued from cover — From the Director

institute was to first clearly define its mission and vision and then recruit the best, most talented faculty whose scientific expertise and research interests closely aligned with LPI's vision of "discovering how to live longer and feel better." The premise behind this simple plan was that great people make a great institute. I was very fortunate to attract and recruit many outstanding principal investigators, almost all of whom are still with the institute today—a testament to their loyalty and strong commitment to LPI. These faculty, in turn, attracted outstanding students, postdoctoral fellows, and research associates and assistants, making the LPI what it is today—an internationally recognized leader in cutting-edge nutrition research on micronutrients, diet, and health.

Together, we have transformed and continue to transform the field of nutrition with mechanism-based research on how nutrients and dietary factors work in the human body at the molecular and cellular level, thereby putting hard science behind nutrition. This knowledge is critical for using nutritional approaches to optimize health and prevent chronic disease, which I believe is the future of medicine and the hallmark of a true healthcare system—helping people to achieve and maintain optimal health, to live with ease and ability, beyond taking care of people when they experience "dis-ease" or "dis-ability."

Fred Stevens

I am pleased to announce that Dr. Fred Stevens will serve as LPI's interim director. Dr. Stevens joined the institute in 2002 and has been a principal investigator since 2005. He is also a professor in OSU's College of Pharmacy. He received his master's degree in pharmacy and his Ph.D. in medicinal chemistry from Groningen University in the Netherlands. Dr. Stevens has been running a highly successful, extramurally funded research program at LPI investigating the role and function of vitamin C and xanthohumol, a compound found in hops, in human health and disease. He is an expert in mass spectrometry-based "metabolomics" research, a comprehensive, "big science" approach for the discovery of biological and health effects of vitamins and dietary phytochemicals in humans. An in-depth profile of Dr. Stevens and his research can be found in the Fall/Winter 2010 Research Newsletter. I am confident that Dr. Stevens will uphold LPI's scientific rigor and focus on transformative research and will successfully maintain the institute's momentum as OSU conducts a competitive national search for a permanent new director, an effort that is already under way.

Anne Glausser

I also would like to introduce you to our latest addition to the LPI team. Anne Glausser joined the institute at the beginning of the year as our new Communications Manager. Anne has a strong background as a science communicator, with a master's degree in Science Writing from the Massachusetts Institute of Technology in Boston, MA. Before joining LPI, she was an Emmy award-winning Health and Science Coordinating Producer with NPR/PBS in Cleveland, OH. Anne already has created a comprehensive strategic communications plan for the institute and is busy implementing this plan, which has dramatically improved our communications efforts. If you haven't already, I encourage you to connect with the institute online, as we share news and updates on a daily basis. You can find LPI on Facebook, Twitter (@LPIatOSU), LinkedIn, and Pinterest (LPInutrition) or subscribe to our blog (tinyurl.com/PaulingBlog). I also encourage you to complete the audience survey included in this issue of the newsletter, which will help in our efforts to better serve your needs and interests and provide valuable insights into how we can most effectively communicate our research findings with the general public.

We have made it a point of pride at the institute to follow in the footsteps of our founder, Dr. Linus Pauling, and share the institute's cutting-edge scientific findings with the public. I hope that you will continue to follow and share the institute's work on the critical importance of diet, micronutrients, and dietary supplements in health promotion and disease prevention, and support the institute as it strengthens and expands its ground-breaking molecular nutrition research and trusted public outreach under the leadership of Dr. Stevens and then, its next, permanent director. Leading the LPI for almost 20 years has been a highly rewarding and productive time in my scientific career, and I am grateful for your support and advocacy of the institute and me personally during my tenure. It's been the opportunity and experience of a lifetime! Thank you! LPI

Continued from cover — Interview with Dr. Arup Indra

Q. What's your role in the Linus Pauling Institute?

A. The Linus Pauling Institute's mission is to achieve optimum health through micronutrients, including vitamins, and a major focus of my research from the beginning has been the role of vitamin A and vitamin A signaling in skin, skin health, prevention of skin diseases, and cancer.

Q. What do you like most about the Linus Pauling Institute?

A. I like its cohesiveness, integrity, and the diversity of scientists involved in different aspects of human health, ranging from metabolomics and aging to diseases like cancer and metabolic syndrome.

Q. What stimulated your interest in skin?

A. Mainly my mentor, Pierre Chambon. One of the things that he asks his students to do is to take one subject and pursue it through the end of your career. I never appreciated that until I started my own lab. When I started in his lab, he mentioned there was a skin project I would be able to work on. Since then, February 1996, I never left working on skin.

Q. What is the structure of skin, and what kinds of cells are found in the different strata of skin?

A. Skin is a very interesting model system in all aspects from lower to higher vertebrates. It keeps the "inside in" and the "outside out," forming a protective layer between you and external insults, whether environmental stresses, allergens, ultraviolet light, and so on. Skin has multiple cell layers, starting with quiescent stem cells, dividing cells, well-differentiated cells, and then finally cells that are terminally differentiated. These terminally differentiated dead cells form an integrated structure with lipids like brick and mortar. That forms a skin barrier, which is critical for keeping us all healthy.

Q. Why does the appearance of skin change as we age?

A. There are two different stages over time—chronological aging and stress responses. As is true for any other organ, aging is inevitable. With chronological aging, our skin structure, function, and responses to the environment change. Skin aging is also due to stresses like carcinogens, solar ultraviolet light, psychological stress, and others. At the molecular level, these changes happen because of the degradation of collagens. Macroscopically, you see drooping, wrinkling, and thinning of the skin.

Q. Do you use rodents or cell cultures to study skin?

A. We use rodent models—preclinical models as human skin equivalents, as well as cell cultures. Essentially, we start with test-tube studies, move into cell culture studies, and then validate those data in rodent models and skin equivalents. Then we move from preclinical models to human studies with our clinician friends at Oregon Health & Science University in Portland or other universities across the nation.

Q. You work on melanoma, which is an often-deadly type of skin cancer. Why is melanoma so dangerous?

A. Melanoma originates from cells called melanocytes, which are the pigment-producing cells in the skin. Melanin produced by melanocytes colors our skin and helps protect against UV radiation-induced damage. But melanocytes are also susceptible to transformation and alteration due to insults from toxic chemicals and UV radiation. If their DNA is damaged and not repaired, they get transformed and may become cancerous. It's very difficult to treat because they're very invasive and metastasize to distal organs. And they're often resistant to traditional chemotherapy and radiotherapy.

Q. Do you think that melanomas that occur in tissues not exposed to UV radiation might be explained mainly by chemical carcinogens?

A. Yes. Melanomas can be formed in parts of the body not exposed to UV radiation. Those melanomas could develop because of exposure to a carcinogen that could cause somatic mutations at different sites or due to inherited genetic mutations that predispose you to melanoma formation.

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Continued from page 3 — Interview with Dr. Arup Indra

Q. Are there specific chemicals that have been linked to melanomas?

A. Yes. There could be a lot of such chemicals; many of them have not been identified. A few common carcinogens have been implicated, including phorbol esters found in certain plants and dimethylbenzanthracene, or DMBA. DMBA can induce mutations in specific genes, such as *ras*, which is mutated in about 60% of human melanomas. Even diet could contribute to melanoma progression. Cooking meat can produce byproducts that get metabolized, and those, consumed in excess, could be a contributing factor for abnormal changes to existing moles on the body.

Q. You characterized the role of a protein called RXR, which is a hormone receptor on the cell nucleus, in the development of skin cancers, both carcinomas and melanomas. What did you find?

A. That was in 2000-2004. We found that the RXR, which is the partner for many receptors, including ones for vitamin A and vitamin D, among others, has a specific but redundant role controlling normal and healthy skin functions. We found that if you don't have these receptors or if the receptors are not responding normally, then inflammation is triggered. The lack of these receptors also leads to degeneration of the hair follicle, resulting in a disease called utriculi in which there is a spontaneous degeneration of hair follicles and lack of hair growth. We showed that rodents lacking those receptors are more susceptible to develop squamous cell carcinoma and aggressive melanoma in the presence of carcinogens.

Q. You showed that in mice specially bred to lack this receptor, there's a higher risk for malignant melanoma after UV radiation.

A. That is right. In patients who don't have these cellular receptors, there is a higher propensity to develop inflammation and lose hair. Specially bred mice without the receptors that are exposed to UV radiation or any other carcinogen develop epidermal tumors, suggesting that this receptor is a tumor suppressor. In addition to epidermal tumors, the mice develop melanomas, but these are indirect effects because the receptors are lost only in specific skin cells called keratinocytes and not in the melanocytes. So the molecular signaling that's going on between keratinocytes and melanocytes is changed, leading to the cancerous transformation of melanocytes.

Q. You also found that the loss of RXR affects the infiltration of immune cells into the skin. How does that process work?

A. As I mentioned, RXR has many functions in multiple cell types. We recently found that the loss of RXR in melanocytes induces a change in immune surveillance. Immunity is very important to prevent cancer progression. What we found is that melanocytes produce factors that direct the body's immune responses and could make an individual more susceptible to developing melanoma. This was a novel finding that we thought was pretty nice because immunomodulation is very important in the prevention of several diseases, including melanoma.

Q. Are human skin cancers associated with the loss of RXR, or is that limited to rodents?

A. I think that's an important question. We validated our observations in rodents and in humans in a publication in 2010. In humans, we found that as a benign mole progresses to an aggressive melanoma and then to a metastatic melanoma, there is a significant loss of RXR in the keratinocytes adjacent to the melanocytic tumors, and we think that is a contributing factor in the pathogenesis of melanoma in those patients.

We found that one of the major challenges in the treatment of melanoma is sustained release of a drug for targeted therapy.

Q. In other work, you found that another protein called CTIP2, which is found in the epithelial tissue and important in the development of skin, the central nervous system, and the immune system, is elevated in human head and neck carcinomas. Does that have diagnostic or therapeutic potential?

A. The CTIP2 protein was initially cloned and characterized in Mark Leid's lab here at OSU. We looked into the expression of the protein in normal skin and diseased skin, including atopic dermatitis and cancer. In collaboration with Gitali Indra, we found that expression of this protein is significantly increased during the progression of head and neck cancer, which is the sixth most common cancer in the world. And it's linked with aggressiveness—it increases as the cancer progresses to a poorly differentiated, more aggressive cancer. We also think that it can serve as a diagnostic marker in rodents and humans. We were granted a nonprovisional patent in 2011.

Q. Is it found in the blood or limited to the skin?

A. It's limited to skin. We did not find it in the blood in our initial studies, but we didn't do a thorough study in blood or in other body fluids.

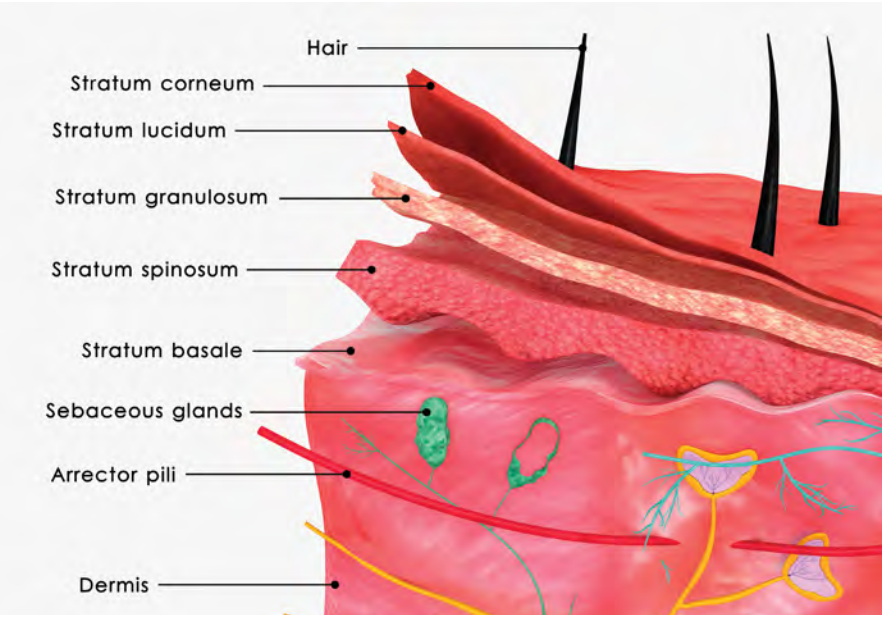
Q. ***You also studied the use of drugs and nanoparticles in the treatment of skin cancer. What are these nanoparticles, and what advantages do they have?***

A. This is part of an ongoing collaborative study. We found that one of the major challenges in the treatment of melanoma is sustained release of a drug for targeted therapy. Slow but sustained release and increased bioavailability are important to treat melanomas and other types of cancers. Our collaborator, Adam Alani, developed lipid nanoparticles that release FDA-approved drugs in targeted areas, such as lymph nodes where melanoma cells usually metastasize. Slow but sustained release of those drugs near the lymph node helps to kill and remove the melanoma cells. In our preclinical models, this has enhanced survival.

Q. ***You worked on another protein called TSLP—thymic stromal lymphopoietin protein—that activates B lymphocytes and is secreted by skin keratinocytes. B lymphocytes secrete antibodies and signaling molecules. TSLP seems to be involved in the initiation and progression of atopic disease. What is atopic disease?***

A. Atopic diseases are inflammatory skin diseases. When the skin barrier is compromised, water is lost from the skin surface, and you're more susceptible to challenges from external insults, such as house mites, dust mites, and allergens, which trigger immune responses. Over time, these immune responses can become systemic and cause atopic dermatitis or eczema.

The new technology shows that measurement of the skin lipids could be a diagnostic tool to distinguish between a healthy and an ill person with compromised skin barrier.



Q. ***So it's a more targeted approach—if you give drugs intravenously, then you don't necessarily get the concentration of the drugs in the lymph nodes where they might be most effective.***

A. That's correct. Giving the drug by injection close to the lymph nodes has been very effective to mitigate disease progression, and we're still working on it.

Q. ***Linus Pauling conducted a series of experiments using UV-irradiated hairless mice that developed a range of cancerous skin lesions. The mice were fed diets without vitamin C or with different amounts of supplemental vitamin C. The researchers found that additional vitamin C significantly delayed the onset of the first lesion and resulted in fewer lesions per mouse.***

A. That sounds very interesting—it seems that there is a lot of evidence that vitamin C has a role. I can tell you that not enough work has been done, probably due to lack of funding, but it's important to consider vitamin C in the prevention of skin cancer.

Q. ***How does TSLP affect atopic dermatitis?***

A. If you disrupt the skin barrier, TSLP levels get elevated. This protein has been found to play a very critical role, and its increase could be one of the earliest events in the pathogenesis of eczema. And one can target that protein and control the inflammation. One has to be careful, though, because TSLP may affect multiple diseases in different organs. Recent studies indicate a surprising role of TSLP in both solid tumors and leukemia. So one has to be cautious—some additional studies are required.

Q. ***Is there any therapy that decreases levels of TSLP? Anything that could be taken orally or could be done to improve the barrier function of the skin?***

A. Yes. One of the major ways you can improve the skin barrier is simply to put back the oil and hydrate. We're currently working on a formulation designed to replenish lipids in the skin and restore barrier functions.

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Q. *Is that applied topically?*

A. Yes, it's topically applied and systemically absorbed. You close down the leaky doors, maintain the skin barrier, and bring down the level of TSLP. That means you can indirectly control TSLP by restoring the barrier with applications of lipids on the skin surface.

Q. *You also made a contribution to skin technology with a new method to measure skin permeability. How does that work, and why is it important?*

A. The new technology shows that measurement of the skin lipids could be a diagnostic tool to distinguish between a healthy and an ill person with compromised skin barrier. Just by looking at the skin lipids in a very fast, reproducible, stepwise fashion, you can determine one's lipid profile. If that doesn't match a standard profile found in a healthy individual, it can be used as a diagnostic tool. If those insufficient lipids are supplemented, the barrier could be restored and the disease progression could be mitigated. This is in the early phase, but we have filed a nonprovisional patent.

Q. *So it hasn't been used clinically yet?*

A. It has not been used clinically yet, but we are confident that it would really work well. We have utilized advanced mass spectrometry for the lipid profiling and found that certain lipids are deficient in eczema patients. I'm very optimistic that this would be the next stage for treatment because we can identify individual lipid profiles of any given individual and therapeutically restore those specific lipids that are altered. This could pave the way to personalized medicine.

Q. *Is it noninvasive?*

A. It's totally noninvasive and extremely efficient. Just put a piece of tape on the skin that takes a few cells from the skin surface. There is no need to draw blood or cut the skin. So, it could be done in individuals of all ages, from children to older people.

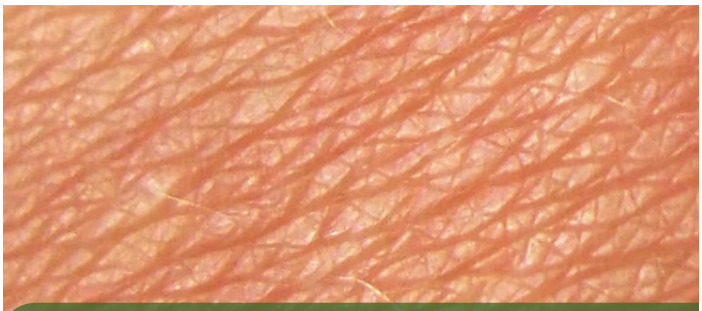
Q. *What kinds of natural molecules are you interested in studying for effects on skin cancers?*

A. We are doing some studies with Fred Stevens at LPI on xanthohumol from hops. We've shown that xanthohumol has multiple roles. It can restore the skin barrier, which is good. Topical applications in a preclinical rodent model of atopic dermatitis reduced skin inflammation, and we are optimistic that it would also mitigate progression of inflammatory skin disease. We think that xanthohumol or its bioactive derivatives has this potential effect. We are also working with Fred on the role of the oil-free seed meal from the meadowfoam plant in the prevention of UV radiation-induced DNA damage, and we have very good results.

In collaboration with Taifo Mahmud in the College of Pharmacy we are also working on other natural products, such as a fish-derived compound called gadusol, which also has a very strong capacity to absorb UV radiation. We have preliminary data indicating it can reduce UV skin damage and reduce sunburn.

Q. *What do you like to do in your free time?*

A. I used to swim a lot, paint, and play a string instrument called the sitar. I was part of an orchestra back in India. During my stay in Europe and as a postdoctoral fellow, I played a lot of sitar and performed in some concerts. Now, I spend time with my son, who's 11 years old. I try to play football with him and teach him math. And he's really interested in science. He loves soccer and American football.



We've showed that xanthohumol has multiple roles. It can restore the skin barrier, which is good. Topical applications in a preclinical rodent model of atopic dermatitis reduced skin inflammation, and we are optimistic that it would also mitigate progression of inflammatory skin disease.

Q. *What will you work on in the future?*

A. I will continue to focus on the tumor microenvironment —how the microenvironment controls everything from stem cell homeostasis to disease progression through inflammation and cancer. I'm positive that the microenvironment affects the pathogenesis of any type of disease, whether it's metabolic disease, inflammation, or cancer. I will continue to pursue the use of these natural products and plant-derived compounds alone or in combination with targeted therapy in the prevention of inflammatory skin diseases and cancer. I would like to see some of these compounds move into clinical trials to improve health and increase the healthy lifespan, in the spirit of Dr. Pauling.

Q. Thank you very much. LPI


Epidemiological Studies

Victoria Drake, Ph.D., Manager, Micronutrient Information Center
and Gerd Bobe, Ph.D., LPI Principal Investigator

Diet has been recognized as an important modifier of disease risk. Nutritional epidemiology is the study of dietary factors that influence the distribution of human disease within a population. Studies in nutritional epidemiology, broadly categorized into **observational/descriptive studies** and **intervention/experimental studies**, investigate the relationship between diet and the occurrence primarily of chronic diseases or other health-related outcomes. Epidemiological data on diet-disease relationships provide clues about the etiology of disease and form the basis of public health recommendations to help prevent and manage disease through nutrition. This article describes the types of studies most commonly encountered to help you better understand the advantages and disadvantages of such studies reported in the media.

Observational/Descriptive Studies

Due to cost, feasibility, and ethical issues, most research in the field is observational/descriptive in nature. Observational studies can be subdivided into *ecologic studies, migrant studies, cross-sectional studies, case-series, case-control studies,* and *cohort studies*. In all of these studies, the association between diet and disease outcome is assessed by simply "observing" rather than "intervening" in what people eat. Dietary or nutrient intake is measured and associated with a health outcome, such as a disease, and a risk assessment is made using statistical approaches. Importantly, observational studies cannot establish causation between a nutritional factor and a health outcome.

In *ecologic studies* and *migrant studies*, average food consumption of a group of people is compared with disease outcomes. While ecologic studies and migrant studies are relatively inexpensive to conduct compared to other studies, a limitation with this approach is that observed relationships between two factors could be caused by a third factor, which is called confounding. Another limitation is called "ecologic fallacy," when associations at the population level do not reflect associations at the individual level. *Cross-sectional studies* measure diet and disease outcome at the same point in time in a study population. The limitation of these studies is that the diet and disease outcome cannot be temporally associated. *Case-series* or *case studies* follow a group with a certain dietary exposure for their disease outcome, with the limitation that case series have no untreated/control group for comparison.

Case-control studies are relatively inexpensive and efficient to conduct and thus seem to dominate the literature in nutritional epidemiology. In a case-control study, diet is usually retrospectively compared in people with (cases) or without (controls) a disease, and a risk for the disease is estimated. Investigators control for many potential confounding variables by matching cases and controls (e.g., age, sex, smoking status), but bias can still occur. Bias is any systematic error in an epidemiological study that results in an incorrect estimate of the association between an exposure and disease risk. There are different types of controls: historical controls; hospital-based controls; and population-based controls, which exhibit less bias. An advantage of case-control studies is that they can account for potential confounders and use data from individuals. The biggest limitation of case-control studies is that they are susceptible to recall and selection bias. Recall bias occurs if the subjects incorrectly recall their dietary history. Recall can also be affected by disease outcome. Selection bias stems from the fact that the most severe cases die before they can be questioned about their dietary exposure or that some groups don't respond to questions about their dietary exposure. *Nested case-control* studies prevent recall bias, as exposure is measured in a healthy cohort/group of people prior (also called prospectively) to the disease outcome. After the disease outcome, cases are matched to controls from this cohort.

To prevent selection and recall bias, large *prospective cohort studies* have been developed. In cohort studies, cohorts or groups of healthy people have been assembled and asked about their diet. Then the cohort is followed over time, and disease outcomes are identified as they develop. Limitations of prospective cohort studies are that they must be large and have long follow-up to get a sufficient number of cases; this can be especially problematic with rare diseases. Residual confounding can still be a problem because people eat a varied diet rather than individual nutrients; thus, it is impossible to isolate the effect of one specific dietary component. Moreover, one food is often replaced by another food with similar macronutrient composition (for example, beans vs. meat as a protein source). A further limitation for all observational studies is dietary assessment. Dietary assessment relies on the ability of individuals to recall complex mixtures of food, whose nutrient content is altered by many environmental factors, such as growing conditions, storage, cooking, etc. Imprecise dietary assessment causes measurement error, as the dietary exposure cannot be accurately measured.

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Intervention/Experimental Studies

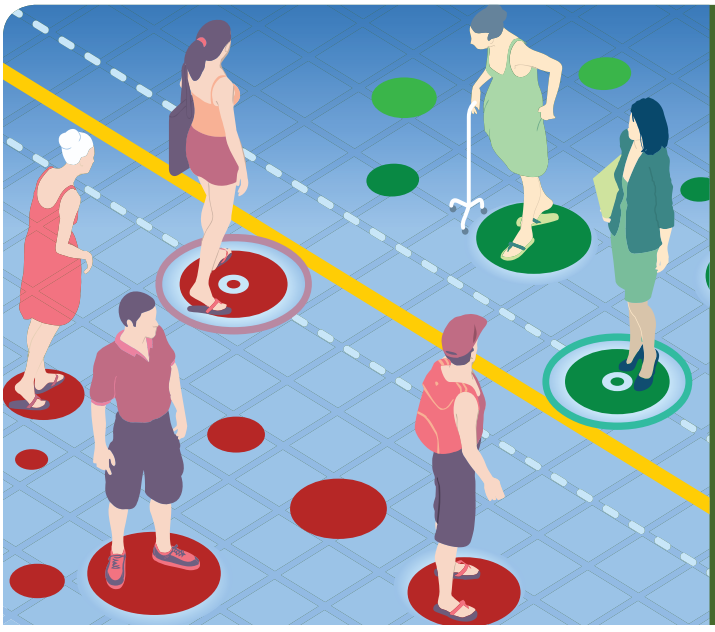
In contrast to observational/descriptive studies, people are assigned a specific dietary exposure in *intervention studies*. In *community trials,* people are assigned the dietary exposure as a group. Community trials are common for studies in children, when certain dietary interventions are assigned to schools where children eat meals together.

In *health promotion studies* and *controlled feeding studies*, people are assigned individually to consume certain food or dietary factors. In health promotion studies, people are encouraged to eat certain food, while in controlled feeding studies, people are provided with certain food. The cost of health promotion studies and, especially, controlled feeding studies is very high; thus, the participants selected have a high risk for disease. Challenges with health promotion studies are low compliance—even the best health promotion studies have a maximal compliance rate of 20-25%. Furthermore, the investigators cannot prevent control subjects from eating food promoted by the intervention. Thus, most studies are too small to detect statistical differences if present. Moreover, studies have to be analyzed based on compliance in addition to group assignment, also called *intention-to-treat analysis*.

Controlled feeding studies avoid the compliance challenge, but the choice of the dietary exposure in controls presents a challenge. Thus, dietary differences between intervention and control are much smaller than what is usually observed in the general population. Moreover, people who participate in these studies are more health conscious than the general population.

Intervention studies, often called *clinical trials*, can be disease prevention or therapeutic trials. A randomized clinical trial has at least one active treatment (intervention) group and a control (placebo) group; participants are chosen for the experimental and control groups at random to reduce potential bias and confounding. A double-blind design is where neither the investigators administering the treatment nor the participants know which participants are receiving the experimental treatment and which are receiving the placebo. A double-blind randomized clinical trial is the "gold standard" of intervention studies but has some inherent problems for studying essential nutrients. Compliance is evaluated by counting pills returned. However, double-blinding is not feasible with dietary intervention studies, as the participants cannot be blinded to what they eat, and evaluating what people eat is not as easy as counting pills. Moreover, the effect of diet on disease outcome is, in contrast to drugs, multifactorial, since diet is a mixture of thousands of compounds and not just a single, active compound. Furthermore, clinical trials with vitamin supplements do not have true placebo groups for comparison; these trials can only assess low versus high intake, unlike drug trials in which subjects in the placebo group do not have any of the tested drug in their bodies.

Results from the various types of epidemiological studies provide information on the role of nutrition in health and disease. *Meta-analyses* and *systematic reviews* pool data from different types of studies and often influence policy or treatment decisions in nutrition, but the heterogeneous nature of the data collected in various studies (e.g., supplement dose, duration of treatment, etc.), as well as the different populations studied, make this challenging. To best understand diet-disease relationships, one must examine the totality of evidence from both observational and intervention studies and also consider data from animal and biochemical studies to evaluate biological plausibility or causality. LPI



DEFINITIONS

Epidemiology: (Greek: *epi*, upon; *demos*, people; *logos*, the study of) the branch of medical science that studies the distribution and determinants of human disease

***Nutritional epidemiology*:** the study of nutritional determinants of disease within a population.

***Bias*:** any systematic error in an epidemiological study that results in an incorrect estimate of the association between an exposure and disease risk.

***Confounder*:** an extraneous factor in an observational study that distorts or biases an association between an exposure and the measured outcome.

Human Genetics and Micronutrients

Alexander Michels, Ph.D., LPI Research Associate

Genes play a large role in how our bodies respond to micronutrients. For any given vitamin or mineral, there is a genetic factor that influences how it functions in the body. These interactions are numerous and can be very complex—even focusing on a particular vitamin like vitamin C and attempting to investigate all of the possible genetic factors that could influence its role in health would result in a dizzying array of possibilities.

This may be one reason that detailed investigations of gene-nutrient interactions are rather uncommon. In a clinical study, researchers often will provide vitamins or some other supplement to a subject and measure a particular outcome, such as the amount absorbed into the blood stream or its impact on enzyme function. When these responses are collected from multiple participants, they can be used to establish the range and average response for the given population. The variation in results is normal and often ignored because a large variety of both genetic (DNA code) and non-genetic (environmental) factors come together in many possible ways to change a response from individual to individual.

With the advent of human genetic sequencing initiatives, such as the human genome project, and sophisticated techniques for measuring the genetic code, more specific information about the interactions between genes and nutrients is now possible. It is becoming clear that some of the "normal" variation observed in nutrition research can be attributed to very specific differences in the genetic code. *Human genetic variation*, proclaimed as the "Breakthrough of the Year" by the journal *Science* in 2007, is a term that is used to describe all the possible differences in the genetic code that may exist among individuals. Because the human genome is large, we are far from discovering all the elements of our DNA that affect how we utilize micronutrients from our diet, but even at these early stages we have some specific information on a few individual genes and nutrients.

Folate

One example of how human genetic variation can affect the interaction between nutrition and health is the B vitamin folate, specifically, alterations in the gene encoding methylenetetrahydrofolate reductase (known as *MTHFR*), which converts one form of folate to another. The function of folate in the body is to mediate reactions that are critical to the metabolism of nucleic acids and amino acids. The body has two forms of folate that participate in these reactions. One form, known chemically as 5,10-methylene-THF, is involved in nucleic acid production, and the other form, 5-methyl-THF, is involved in methionine production along with vitamin B_{12}. The enzyme *MTHFR* converts the methylene form of folate to the methyl form and thus serves as a mediator between the two fates of folate.

There are two common genetic variants of *MTHFR*, known as 677C and 677T. These variants are the results of a single nucleotide polymorphism or SNP, one of the most common variations in the human genetic code. A SNP is a change in a single base pair (adenine-thymine and guanine-cytosine that create the rungs on the DNA double helix) of a gene. Although it is only one change in hundreds or thousands of DNA bases that make up a particular gene, even a relatively small change can have a large impact, depending on the site of alteration within the gene. In the case of *MTHFR*, the SNP that changes 677C to 677T results in decreased activity of the enzyme.

Since everyone has two copies of the *MTHFR* gene, the effect of the variant can vary from person to person by the different combination of the variants. People who have one or more copies of the 677T have less *MTHFR* activity in their cells compared to those who have only the 677C. These decreases in *MTHFR* activity have been linked with a higher risk of low folate status, higher levels of homocysteine in the blood, and increased risk for certain types of leukemia. However, these effects depend heavily on folate nutritional status—if folate consumption is high, the genetic variant seems to have less effect.

Although it is tempting to label the 677T variant in *MTHFR* activity as negative, there are possible benefits to these genetic variants. If the cell has less active *MTHFR*, it would have larger amounts of 5,10-methylene-THF that can help support the production of DNA and RNA. There is some speculation that this may protect the body in certain circumstances. For example, a person with the variant may have a lower risk for cancer or congenital defects when the levels of folate in the diet are very low.

Vitamin C

Another example of genetic variation affecting micronutrients is vitamin C. The absorption of vitamin C from the gut into the blood stream and its distribution throughout the body is governed by two proteins, aptly named the sodium-dependent vitamin C transport proteins, SVCT1 and SVCT2. These proteins are immensely important in maintaining high levels of vitamin C in the blood and preventing all its loss in urine.

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Continued from page 9 — Human Genetics and Micronutrients

"With the advent of human genetic sequencing initiatives, such as the human genome project, and sophisticated techniques for measuring the genetic code, more specific information about the interactions between genes and nutrients is now possible."

Genetic variation can take on many different forms, from subtle differences in the genetic code to severe changes in DNA structure. The effects of these genetic changes can also be quite variable. Severe forms of genetic variation result in genetic diseases, such as Huntington's disease, Tay-Sachs disease, or sickle-cell anemia, but more common are the less severe genetic changes that often have subtle effects on health. Some of these variants are reported as "gene mutations," but the term "genetic variant" is more descriptive because the effects of these changes are not unequivocally negative or positive.

As is the case with many genes, SNPs appear in the human genetic code, affecting both SVCT1 and SVCT2, but most of them are rare and/or do not have any noticeable impact on human health. However, as recently reviewed by researchers at the Linus Pauling Institute, three SNPs present in the gene encoding SVCT1 and at least one SNP present in the gene encoding SVCT2 have been associated with lower vitamin C levels in the blood, even when the amount of dietary vitamin C consumed is about the same as in individuals without these SNPs.

Furthermore, the genetic variants in the SVCT proteins have been associated with changes in health status or increased risk of chronic disease. In some study populations, genetic variants have been associated with an increased risk of preterm birth of infants, Crohn's disease, periodontal disease, lymphoma, and gastric cancer, but it isn't clear if the disease risk is directly attributable to some unknown effect of these genetic changes or a direct change in vitamin C levels in people who have these genetic polymorphisms.

Not all of the SNPs present in the SVCT1 and SVCT2 proteins are rare. In fact, some are quite common in Caucasians and African Americans. If these genetic variants alter the way our bodies absorb vitamin C, how it accumulates in tissues, and how it is excreted from the body, there may be profound implications in vitamin C research, including how much vitamin C we recommend for daily intake. However, despite the possibility that these polymorphisms in a study population may radically affect the interpretation of results, an investigation of the genetic variants in individuals participating in vitamin C studies is rarely, if ever, done.

Haptoglobin

A third example of genetic variation is the relationship between iron metabolism and a protein called haptoglobin. The function of haptoglobin is to bind to the hemoglobin in red blood cells that is released when these cells are damaged or destroyed. Haptoglobin prevents iron released from the hemoglobin from causing harmful reactions in the blood and also binds to the iron in order to prevent invading pathogens from using it to grow, thus limiting the spread of bacterial infections.

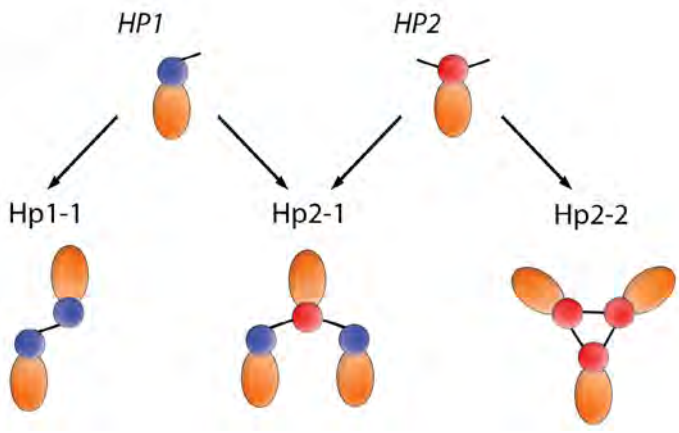
The haptoglobin gene has genetic variants in humans, but unlike *MTHFR* and SVCT discussed above, this variation is due to a repeated sequence of the genetic code. In other words, one variant of the haptoglobin gene—called Hp2—has a section that is duplicated and makes it twice the size of the other variant, known as Hp1. The protein produced by the Hp2 variant functions a little differently from Hp1—it creates large complexes of haptoglobin that do not bind hemoglobin as effectively. It is thought that people with Hp2 may have a higher degree of oxidation reactions in their blood due to the poor control of iron. Thus, they may require more of the antioxidant vitamins C or E in their diets to compensate, at least compared to people with only the Hp1 variant.

However, there may be other effects to having a variant haptoglobin. People with the Hp1 variant seem to be more susceptible to some infectious diseases, especially malaria. These variants may also play a role in anemia risk when iron status is low, but the exact relationship is not well defined. Another haptoglobin form, Hp2-2, is associated with an increased risk for atherosclerosis in diabetics. Studies have suggested that vitamin E supplementation may help prevent or attenuate atherosclerosis in diabetics with Hp2-2 but not in diabetics without Hp2-2. Thus, it may be difficult to correctly interpret the results of some vitamin E supplementation studies if genetic variants in haptoglobin are not identified.

Many more genetic variants are being discovered that have roles in nutrition. As this list grows, more research is needed to estimate their impact on health and disease.

> This is a field that has potential for an enormous effect on recommendations for public health.

This is a field that has potential for an enormous effect on recommendations for public health. An exciting possibility is that someday a full understanding of variations in the genetic code might lead to personalized recommendations on how to eat right and take dietary supplements for good health. LPI



Hp1 *has the highest binding affinity for iron but is associated with greater susceptibility for malaria*

Hp2 *is associated with higher oxidative stress and increased risk for atherosclerosis in diabetics*

IN MEMORIAM

Sharon Krueger

At LPI, Sharon will be remembered for her academic diligence, optimistic attitude, courage, and abiding love of cooking and nature.

A celebration of the life of Dr. Sharon Krueger (1960-2015), Research Assistant Professor since 2008 in the Linus Pauling Institute, was held on February 11 in the Linus Pauling Science Center. Sharon died on December 27, 2015, following an accident at her home.

A native of Wisconsin, Sharon moved to Corvallis in 1987 to earn her doctorate at Oregon State University. She became a postdoctoral researcher in LPI Principal Investigator Dave Willams's laboratory, where she worked extensively on the flavin-containing monoxygenases (FMO), compounds that metabolize drugs, plant alkaloids, and toxins, including pesticides. One of Sharon's over 30 scientific papers is the most-cited publication in the literature on FMOs. Over her long association with Dave Williams, Sharon also worked on the protection by indole-3-carbinol in cruciferous vegetables against cancer in mice caused by exposure to the ubiquitous environmental carcinogens, polycyclic aromatic hydrocarbons, formed by the burning of carbon fuels. In his remarks at her service, Dave praised Sharon's "grit and determination" and credited her with sustaining for 24 years a continuously funded grant from the National Institutes of Health.

More recently, Sharon had taken on responsibility for clinical research coordination in LPI and served as the research coordinator for OSU's Superfund Research Center. Sharon mentored many undergraduate students and also played an important role on the Commission on the Status of Individuals with Disabilities. For many years, she had coped with a rare genetic disease that causes metabolic muscular dystrophy. As OSU's Gabe Merrell said, Sharon became a very effective campus advocate for accommodating people with disabilities.

At LPI, Sharon will be remembered for her academic diligence, optimistic attitude, courage, and abiding love of cooking and nature. As LPI's Director Balz Frei noted, "What was so impressive and inspiring about Sharon was how she dealt with her debilitating disease. She didn't let that disease define her or slow her down. She was fiercely independent...she lived her life to the fullest...had an amazing work ethic...and brought her wonderful spirit and positive attitude to work every day."

She is survived by her mother, sister, and two brothers, one of whom—Dan—spoke at her service about their childhood on a Wisconsin farm growing ginseng and eating freshly picked vegetables. LPI

Epidemiology and Personal Risk

Gerd Bobe, Ph.D., LPI Principal Investigator

We hear every day about new studies reporting that some nutrient or micronutrient either causes or protects against a disease. Often, the evidence is cyclical—for a decade fat was dangerous, now sugar is dangerous, and fat not as bad. Is science like fashion, going in and out of vogue?

The science that leads the controversy is epidemiology. According to Leon Gordis, a leading epidemiologist, "Epidemiology is the study of how disease distributes in populations and what factors influence or determine this distribution." Epidemiology rose to prominence in the 1800s with industrialization. Physicians studied what factors influenced who got sick from infectious diseases like cholera or tuberculosis. People learned from epidemiologists how diseases spread and how one could protect oneself from those diseases. It was detective work to determine the source of an outbreak. Nowadays, we often hear of this kind of research in the case of a foodborne illness to determine its cause. We have surveillance systems and preventive and control programs like the Centers for Disease Control and Prevention to prevent outbreaks of infectious/transmissible diseases.

The rise of "chronic disease epidemiology" began in the 1950s. Chronic disease epidemiology is closely tied to the emergence of smoking as a risk factor for cancer and cardiovascular disease. For years, the tobacco industry fought the association between smoking, lung cancer, and other diseases, but the accumulated evidence is now overwhelming. In contrast to infectious diseases, chronic diseases develop over decades; the cause and effect connection is less evident.

The link between smoking and lung cancer is a good example to explain risk. "Lifetime risk" (i.e., absolute risk) is the proportion of people who will develop a disease during their lifetime. For example, a 2006 European study found that the lifetime risk to develop lung cancer for current male smokers was 15.9%. In other words, about 16 male smokers in a population of 100 male smokers are likely to get cancer related to smoking. In comparison, the lifetime risk for men who never smoked was 0.2% (less than one person in 100). Often these numbers are expressed as the proportion of diseased people within a population (e.g., cases per 1,000 people). Absolute risks are not often available for the whole population, so epidemiologists calculate risk in the people they follow. People are usually followed for a certain time period, and incidence rates describe the risk to develop a disease within a certain time period. For example, five-year survival rates for cancer estimate the proportion of people who will survive for at least five years after diagnosis. Since people choose to stay in studies for different time periods, epidemiologists calculate "person-times" (usually years) to more accurately estimate risk. For example, epidemiologists calculate "package-years" of smoking—the number of packages of cigarettes a smoker consumed over the course of many years.

For lifestyle factors, we usually weigh our risks; we compare the risk for two different choices, which gives "relative risk." In our example, the relative risk of developing lung cancer when comparing current male smokers to males who never smoked is 15.9% divided by 0.2%, or 79.5%. So, men have a 79.5% higher relative risk to develop lung cancer if they smoke compared to men who never smoked. Often, we weigh our risk. A relative risk of 1.0 indicates equal risk (e.g., no difference in disease risk between smoking or not smoking), numbers lower than 1.0 indicate decreased risk, and numbers higher than 1.0 indicate increased risk. For calculating relative risk, one has to have an estimate for the absolute risk in the population, which is often unavailable. In that case, epidemiologists calculate the odds ratio—what is the ratio of the odds that an exposed person develops the disease versus the odds that a nonexposed person develops the disease. In our example, the odds ratio of developing lung cancer when comparing current male smokers to those who never smoked is [15.9/(100-15.9)]/[0.2/(100-0.20)] = 94.3%. So, men have 94.3% greater odds to develop lung cancer if they smoke compared to never having smoked. Hazard ratios compare the risk to develop a disease during a specific time period (person-years) between exposed and nonexposed people.

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Men have a 79.5% higher relative risk to develop lung cancer if they smoke compared to men who never smoked.

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As you can see, the estimators of risks try to predict the absolute or relative risk to develop a disease for an average person. Thus, your personal risk may be higher or lower depending on other factors. Furthermore, when comparing relative risk estimates, one always has to consider the absolute risk. Smoking and cancer has gotten so much attention because the absolute risk of male smokers to get lung cancer is very high (15.9%), and the absolute risk of men who never smoked is very low (0.2%). To better show that smoking is such an important risk factor for lung cancer, epidemiologists calculate "attributable risk," which is the number of current smokers in the total lung cancer cases, and the "attributable relative risk," which is the proportion of current smokers in total lung cancer cases. The attributable risk is used to estimate how many lives could have been saved if people did not smoke. Clearly, those people would then die of other causes, and other lifestyle factors can be associated with those deaths.

While smoking is now unequivocally considered a risk factor for disease and premature mortality, other lifestyle and dietary factors are still very much debated. Currently, sugar and salt are the most hotly debated. Sugar and salt consumption have increased over the last decades, coinciding with the obesity epidemic in the U.S. and the rest of the world. Are sugar and salt to blame for the obesity epidemic? Do we need governmental policies to restrict sugar and salt added to processed food? Should we have a salt and sugar tax? Policy makers look to epidemiologists for guidance in answering these questions. Epidemiologists examine the association between chronic diseases and dietary habits and calculate if certain dietary factors are associated with disease risk. To provide guidance for policy makers, epidemiologists combine results of many studies in meta-analyses to determine if there is a general significant trend to link a disease with a specific dietary factor.

Of course, each person wants to know about their personal risk. We eat differently, have different disease histories in our families, and have different behaviors. What constitutes a risk for one person may not be a risk for another person (e.g., sugar and diabetes). Furthermore, what is relatively safe during one stage of our lives can become a risk at a later stage of life (e.g., pneumonia, when immune systems are weakened). For example, the risk of developing colorectal cancer is 1 in 20, or 5%, but goes up to 18% for people with chronic inflammation in their large intestines and is nearly 100% for people with a genetic predisposition to familial adenomatous polyposis (FAP). These people will develop colorectal cancer in their forties unless their large bowel is removed.

So, people have different risk profiles based on their genes, their physiology, and their environment (e.g., diet, exposure to second-hand smoke and carcinogens, etc.). To help evaluate risk, epidemiologists have developed risk assessment tools, such as the Breast Cancer Risk Assessment Tool from the National Cancer Institute. Some women have elected to have mastectomies because of a family history of and genetic predisposition to breast cancer. We have all heard of people who died of cardiovascular disease or cancer although they ate healthily, exercised regularly, and got regular screening exams. Fortunately, there are genetic cancer risk counselors available for those who know they have an increased risk of hereditary cancer.

> "Our next frontier is personalized cancer prevention for cancer survivors. As more and more patients survive longer from cancer, we will learn more about how to help them."

People with an increased risk of hereditary cancer often form support groups to learn how they can decrease their risk. Since their risk profiles are very different from those of the general population, they are more likely to take medication, which may have some side effects, to decrease their risk (e.g., tamoxifen and raloxifene for breast cancer prevention). Some of the drugs may themselves pose potentially lethal risks, which, however, are outweighed by the genetic cancer risk (e.g., celecoxib and sulindac for people with FAP).

Our next frontier is personalized cancer prevention for cancer survivors. As more and more patients survive longer from cancer, we will learn more about how to help them. We will learn more about how genetic polymorphisms (changes in the DNA code) affect the risk of developing, for example, FAP and how they affect cancer risk. As we target cancer treatment based on the genetic profile, we will learn more about how our genetic profile interacts with our lifestyle choices to either increase or decrease our disease risk. Studies that identify and characterize patient responders to specific chemopreventive treatments, including dietary choices, will help us to advance personalized cancer prevention.

Epidemiology is beginning to diverge into two public health branches. One branch provides guidance for policy makers and medical/nutritional societies in making recommendations for the general population. The other branch applies successes in cancer treatment to personalized disease prevention, allowing individual recommendations tailored to a person's familial and environmental risk profile.

DEVELOPMENTS

This year marks LPI's 20th anniversary at Oregon State University. The Institute has grown substantially since its move to OSU and now has 12 Principal Investigators, 4 adjunct faculty, 14 research staff, and 6 graduate students, all working together to make important discoveries in nutrition that benefit our health. Please help to celebrate the continuation of Linus Pauling's legacy by making a gift that honors Dr. Pauling and all the members of LPI who have joined in our efforts over the years. You can make a donation online at http://lpi.oregonstate.edu/giving-linus-pauling-institute.

We encourage you to keep up with our research and other activities by visiting LPI's website (http://lpi.oregonstate.edu), our Facebook page (https://www.facebook.com/LinusPaulingInstitute), and our blog (http://blogs.oregonstate.edu/LinusPaulingInstitute).

And we are here to help answer your questions.

Have a happy summer!

LPI 20TH ANNIVERSARY

1996 ~ OREGON STATE UNIVERSITY ~ 2016

~ OBER TYUS ~

IN MEMORIUM

Ober Tyus, who served as LPI's Development Director from 1996 through 1999, died on February 29, 2016. Ober was born in Philadelphia on June 2, 1946, and earned undergraduate and graduate degrees from the University of Georgia. Early in his career he worked for Disney and later had fund-raising positions at Rollins College in Winter Park, Florida, and Pacific University in Forest Grove, Oregon, before assuming the fund-raising position with LPI. Ober spent many days at the Linus Pauling Institute of Science and Medicine in Palo Alto, California, reviewing the Institute's fund-raising activities and provided invaluable help when the Institute moved to Corvallis to become part of Oregon State University in the summer of 1996.

Ober was an Anglophile renowned for his deep knowledge of the British monarchy. He was also a talented actor and over the decades played various roles in small theater productions. He was very active in the Atlantic Center for the Arts, located in New Smyrna Beach, Florida, where he lived for many years. Ober loved to travel and continued his adventures in the decade since his retirement, as well as consulting work for various nonprofits and other individuals and organizations.

Ober's larger-than-life presence will be missed by all of those who knew him and treasured his indefatigable work for LPI. LPI

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Look for these informative articles inside!

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A "life-income gift" guarantees an income stream for life. Additional benefits may include:

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To learn more about gifts that pay income, **please go to http://bit.ly/1PAAbIM**, or call me at **800-354-7281** for a personalized illustration.

Sincerely,
Jeff Comfort, Vice President, Principal Gifts and Gift Planning

Special thanks to Barbara McVicar for editorial assistance and photographs and to authors of signed articles.