SPRING / SUMMER 2010

OSU Oregon State University

The Linus Pauling Institute

RESEARCH NEWSLETTER

From the Director

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

As director of LPI, I am often asked what supplements I take—after all, thinking about and researching micronutrients every day, I should know what dietary supplements are most important. While I think eating a healthy diet, exercising regularly, maintaining a healthy body weight, and avoiding tobacco are of utmost importance to maintain good health, I also think that some dietary supplements are critical for optimum health.

First, I take a daily multivitamin/mineral with 100% of the Daily Value (DV) or Recommended Dietary Allowance (RDA) of most vitamins and essential minerals. I take this multivitamin primarily as "health insurance"; that is, to make sure I get at least the recommended amounts of most vitamins and minerals in case I don't get each and every one of them from my daily diet. This multivitamin is not an excuse not to eat a healthy diet; instead, I consider taking a multivitamin part of a healthy lifestyle. Plus, it's dirt cheap—you can buy a year's worth of multivitamins for less than a nickel a day!

Next, I take 500 mg of vitamin C every day, in keeping with LPI's recommendation to get at least 400 mg daily, which should be enough to saturate my vitamin C body pool. I also get 60 mg of vitamin C from my multivitamin and another 100-200 mg from my diet, including a glass of fresh grapefruit juice every morning and additional fruit and vegetables as part of my regular diet. I take the free acid supplement of vitamin C—ascorbic acid—as I don't have any stomach or gastrointestinal problems with its acidity; but if you do, try a mineral salt of vitamin C like calcium or magnesium ascorbate.

continued on page 2

Vitamin D Gets An "A"

An Interview with Adrian Gombart, Ph.D.
Associate Professor of Biochemistry
and Biophysics
LPI Principal Investigator

Q. When did you decide on a career in science?

A. I was interested in science when I started college, and I was thinking about becoming a veterinarian. After gaining some practical experience in vet clinics and not getting into vet school the first time I applied, I grew impatient and applied for a master's program in genetics at Oregon State University, where I was an undergraduate. I completed the program and published two papers on baculovirus research with George Rohrmann. By that time I was pretty convinced that I didn't want to go to vet school anymore; I wanted to pursue a career in research. I earned my Ph.D. in microbiology at the University of Washington in Seattle and went to Los Angeles for postdoctoral work.

Q. What brought you to the Linus Pauling Institute?

A. I had been in Los Angeles for 15 years working at Cedars-Sinai Medical Center. My family and I enjoyed Los Angeles, but it wasn't really a place where we wanted to stay permanently. Near the end of my tenure in LA, my research had started to focus on vitamin D and its effect on the innate immune system. We made a very exciting discovery that resulted in an NIH grant award, which gave me the opportunity to start looking for positions elsewhere. It turned out that there was a position open in the Linus Pauling Institute. I knew that LPI had moved to Oregon State and that it was well known for excellent research. My own research dovetailed nicely with LPI's research programs, and I felt that it would be a really good match to bring our vitamin D research on immunity to LPI's Healthy Aging Program.

continued on page 3

Continued from cover — From the Director

I also take a daily supplement of 2,000 IU of vitamin D, which is in addition to the 400 IU I get from my multivitamin. Vitamin D deficiency is a major health problem in the U.S. The majority of Americans have serum 25-hydroxyvitamin D levels less than 32 ng/mL, putting them at increased risk for osteoporosis and bone fractures and possibly hypertension, several cancers, and multiple sclerosis. In addition, the work by LPI's Fritz Gombart has shown that adequate vitamin D intake is essential for healthy immune function and protection against infectious diseases like tuberculosis. I had my serum vitamin D level checked a little over a year ago, and to my dismay it was barely sufficient at only 30 ng/mL. The doctor put me on 50,000 IU of vitamin D per week for 8 weeks to load up my body stores for the winter, and now I am on a maintenance dose of 2,000 IU. This dose also is consistent with the Institute's recommendation, which is higher than the government's recommendation of 400 IU (rumored to be increased soon to 1,000 IU).

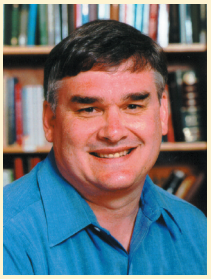
Furthermore, I take a daily 1,200-mg fish oil supplement, which contains 360 mg of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These omega-3 fatty acids are important for cardiovascular health and have been shown to lower the risk for heart attacks and sudden cardiac death. In addition, they have strong anti-inflammatory properties. Chronic inflammation increases with age and is a critical contributing factor to many chronic diseases, including heart disease and certain types of cancer and neurodegenerative diseases. Omega-3 fatty acids also are important for brain health, and accumulating evidence suggests that DHA may slow cognitive decline in older adults and lower the risk for Alzheimer's disease and other types of dementia. And some very recent evidence suggests that fish oils may help maintain the ends of DNA called telomeres. Telomere shortening has been suggested to be an indicator of biological aging and may be associated with an increased risk of heart disease.

Finally, I take 400 mg of lipoic acid and 1,000 mg of acetyl-L-carnitine (ALCAR) daily. This is based on the research by LPI's Tory Hagen on the role of these "age-essential" micronutrients in improving mitochondrial function and energy metabolism with age, and the research in my own laboratory indicating that lipoic acid has anti-inflammatory properties and lowers body weight and serum triglycerides in experimental animals. In addition, lipoic acid is well known to stimulate the insulin receptor and improve glucose metabolism, and is used in Europe to treat diabetic complications.

My lipoic acid-ALCAR supplement also provides 300 mcg (100% of the DV) of biotin and 150 mg (15% of the DV) of calcium, which complements my multivitamin that contains only 30 mcg of biotin (the current RDA) and 200 mg of calcium. Multivitamins do not contain the daily recommended calcium dose of 1,000 mg because it would make the tablets too bulky. Therefore, it is important to make up the difference with an extra calcium supplement or from your diet. I close the gap from the 200 mg in my multivitamin with 150 mg from the lipoic acid-ALCAR supplement and my daily 16-oz cappuccino—providing about 250 mg of calcium—and an 8-ounce low-fat probiotic yogurt with another 400 mg.

My daily supplements in combination with my diet and regular exercise closely reflect LPI's Rx for Health (see http://lpi.oregonstate.edu/lpirx2.html), except that I don't take a supplement of 200 IU natural source alpha-tocopherol (*d*-alpha-tocopherol). I get 30 IU of vitamin E from my multivitamin and additional vitamin E from my fish oil supplement. Since I have experienced an increased tendency to bleed if I take both fish oils and extra vitamin E, I do not take the latter on a regular basis. My health is excellent, and I hope to remain healthy for a long time. Our Rx for Health is designed to help you live longer—with the best health, too. LPI

Honors for LPI Faculty



On September 23, 2009, Dr. David Williams, an LPI Principal Investigator and Professor of Environmental and Molecular Toxicology, was honored with the OSU Alumni Association Distinguished Professor Award. The award is given for "outstanding professional achievement through teaching and scholarship, service to the university and the community, and professional leadership, nationally and internationally."

Dr. Balz Frei, LPI's Director and Professor of Biochemistry and Biophysics, was named an OSU Distinguished Professor on January 26, 2010, for achieving "national and international stature as a result of [his] contributions to scholarship/creative activity, research, education, and service, and whose work has been notably influential in [his] fields of specialization."

Congratulations to our Distinguished Professors!

Continued from cover — Interview with Dr. Adrian Gombart

Q. Did you know much about Linus Pauling?

A. I knew that Linus Pauling was an Oregonian and had attended Oregon State University. His name came up frequently because of his scientific stature, especially his Nobel Prize-winning work on the chemical bond and protein structure. I was aware of his Nobel Peace Prize, too.

Q. What do you like about the university environment?

A. The university is a very collegial environment that promotes good interactions among researchers with very different interests. I think that happens more here than in medical centers. The university is a little less political—people are more interested in achieving their research goals rather than protecting their territorial ambitions. That being said, I have some great collaborators in Los Angeles, and our projects are still ongoing and productive.

Q. Are you always thinking about science or do you have other interests as well?

A. I think about science a lot! It's what I really enjoy, but I do have other interests, like travelling. I enjoy activities with my family—my wife and three boys—like walking, biking, basketball, and other sports. My kids play organized sports, and we spend the weekends running around to practices or games, too. Also, I enjoy reading, music, and movies.

Q. What obstacles do scientists often confront?

A. Time management! Balancing family and social life with your career is very challenging. I haven't done much classroom teaching, but I think I will do more now that I'm at a university instead of a medical center. I feel that my time will be spread even thinner with that additional activity, but I find it enjoyable.

Q. What about competition for grants?

A. Competition for grants is difficult. The funding continues to be flat or even decreased because of inflation. It's a critically important issue, though, because you have to continue to get funding to be successful.

Q. Early in your career at the University of Washington, you investigated the genetics of viruses, especially measles. What did you learn from that work?

A. Despite the measles vaccine, measles is still a major killer, mainly of children in developing countries, and a lot of that has to do with nutrition. If you are malnourished, you don't fight infections very well. The vaccine for measles, while quite effective, is not effective enough to eliminate the disease. Measles is one disease like polio that could potentially be eliminated because humans are the only reservoir. There is still interest in improving the vaccine and understanding the biology of the virus. I learned some interesting basic biology from studying the virus, especially something called RNA editing that is used by the measles virus to increase the number of proteins that its RNA genome can code for by making changes to the messenger RNA sequence. Various iterations of RNA editing are used by higher organisms, as well. At the time, it was a very intriguing mechanism.

Q. Does that make it more virulent?

A. Yes, the process produces a protein that is probably really important for gene transcription—creating RNA copies from the genome. Subsequent research findings suggested that the protein made by RNA editing contributes to disease severity. The virus lacking the protein would be considered less virulent.

Q. Somewhat later when you worked at UCLA, you got involved in cancer research and studied mutations to tumor suppressor genes and their functional effects. How do tumor suppressor genes work?

A. In cancer cells there are oncogenes and tumor suppressor genes. The oncogenes are considered the accelerator—they promote the growth of the tumor when they become altered. So overexpression of oncogenes pushes cell proliferation. The tumor suppressor genes are like the brakes in the cell—they prevent cells from growing too rapidly or even growing at all.

Q. Do you find tumor suppressor genes in all cells?

A. Yes. Quite a few have been discovered, including p53, retinoblastoma, and inhibitors of cyclin-dependent kinases. Cyclin-dependent kinases are important for progression through the cell cycle. If this cycle stops, then the cell can't grow. The cyclin-dependent kinase inhibitors put brakes on progression through the cell cycle.

Q. Is cancer always associated with altered activity of tumor suppressor genes?

A. Yes, all cancers have some tumor suppressor dysfunctions, and p53 is most commonly affected. Also, deregulation of the pathway in which the retinoblastoma tumor suppressor protein functions is very common in most cancers.

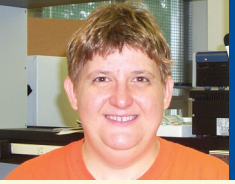
Q. Are there ways to influence the activity of tumor suppressor genes in cancer cells?

A. There are a number of ways to turn them on. Chemotherapeutic agents damage cancer cells, leading to an increase in the expression of p53 that inhibits tumor cell growth.

Q. Are there nutritional strategies to help prevent cancer by influencing the activity of tumor suppressor genes?

A. Yes, histone deacetylase inhibitors induce a cyclin-dependent kinase inhibitor called p21. This stops cells from growing. Rod Dashwood's and Emily Ho's groups in LPI are working on organoselenium and sulforaphane compounds found in garlic and broccoli that act as histone deacetylase inhibitors. Also, there is great interest in using vitamin D and vitamin A compounds to induce the tumor cells to change their properties and stop growing. Vitamin D induces the expression of p21.

continued on page 5



Preventing Cancer with Clay

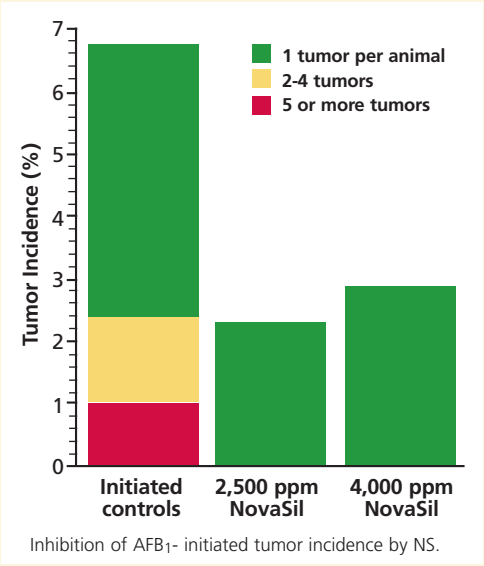
Gayle Orner, Ph.D.
Assistant Professor (Senior Research)

Aflatoxins are naturally occurring toxins formed by molds. Corn and peanuts are often affected, especially when grown in drought conditions or stored at high temperature or humidity. These toxins can produce severe liver damage or cancer in humans. Even with the best agricultural practices, some contamination by aflatoxins, such as aflatoxin B_1 (AFB_1), is unavoidable. In the United States, strict regulations and testing by industry and government agencies have been largely effective at minimizing aflatoxin levels in the nation's food supply, though at a cost of approximately 1.4 billion dollars per year. However, the screening process is not foolproof. Despite the fact that raw materials are screened, every few years an outbreak of fatal aflatoxicosis occurs in dogs, a species far more sensitive than humans to the acute toxic effects of aflatoxins. The same company implicated in the recent peanut butter *Salmonella* outbreak had previously been cited by the FDA for distributing peanut products containing unacceptable levels of aflatoxins on multiple occasions. In many developing countries, the process of screening and discarding contaminated grains is just not economically feasible. Worldwide, over 4.5 billion people are unavoidably exposed to chronic, high levels of aflatoxins, and this exposure is believed to be a major factor in the high rates of liver cancer in some countries. There is an urgent need to develop safe and effective methods to reduce the impact of accidental or unavoidable aflatoxin exposures on human health.

One strategy to minimize dietary aflatoxin exposure is to include substances in the diet that bind to these agents and slow their uptake. LPI investigator Dr. George Bailey and colleagues have demonstrated that the successful cancer chemopreventive agent chlorophyllin (CHL) acts primarily through this mechanism. Another agent that appears to block aflatoxin uptake through a similar mechanism is a type of clay known as NovaSil (NS).

Geophagy, the deliberate consumption of dirt or clay, has been a well-documented phenomenon for centuries. One hypothesis for the consumption of clay by certain tropical birds is that the clay reduces absorption of plant toxins and allows the consumption of plants that would otherwise be toxic. Native American recipes for acorn bread frequently included mixing the meal with clay and water, a practice that reduced the levels of bitter-tasting tannins. In some cultures, geophagy is common among pregnant women who report that the practice reduces the nausea and vomiting that is common in the first trimester. This may be due to the ability of the clay to adsorb toxins, buffer the gastrointestinal tract, or provide supplemental calcium. Other clays might provide protection towards toxins like aflatoxins.

NovaSil is an anti-caking additive commonly added to animal foods. In the late 1980s our collaborator, Dr. Timothy Phillips from Texas A&M University, first reported that this particular clay bound to aflatoxin with high affinity and high specificity. His research team subsequently reported that NS could prevent the acute toxicity induced by aflatoxin in a wide variety of animals, including rats, mice, chickens, turkeys, cattle, lambs, and pigs. In poultry, NS was so effective that it was able to provide almost complete protection against poisoning from aflatoxins even when the AFB_1 levels were hundreds of times above the toxic levels. Recently, Dr. Phillips' laboratory found that supplementation with NS reduced levels of aflatoxin biomarkers in the blood and urine of humans from Ghana who are exposed to high levels of aflatoxin in the diet.



Inhibition of AFB_1- initiated tumor incidence by NS.

Although these studies clearly showed that dietary clay could protect against the acute toxicity of aflatoxins, there was no direct evidence that NS could prevent cancer in any animal model. In 2006, I received an LPI pilot project grant to determine if NS could prevent aflatoxin-induced DNA damage and cancer in rainbow trout. Trout were selected for this project for several reasons. Trout are exquisitely sensitive to aflatoxin-induced liver cancer; indeed, the first evidence that aflatoxin caused cancer was from studies conducted in the 1960s by OSU researchers using rainbow trout. Studies involving hundreds of animals can be conducted in this model at a modest cost. There is a long history of using rainbow trout to study chemopreventive agents, including the seminal work by Dr. George Bailey's laboratory that established CHL as a highly effective chemoprotective agent against aflatoxin-induced liver cancer.

Two separate tumor studies were conducted to determine if NS inhibited AFB_1-induced DNA damage and liver cancer. In the first study, trout were given diets containing AFB_1 at three times the level allowed in U.S. food supplies, plus one of two levels of NS or CHL. In each study, NS significantly reduced the amount of AFB_1 bound to liver DNA. The figure above shows the effect of either 2,500 or 4,000 ppm NS at inhibiting the liver tumor incidence induced by 60 parts per billion (ppb) AFB_1. NovaSil and CHL had comparable effectiveness in inhibiting tumor incidence induced by 160 ppb AFB_1 (not shown).

Our pilot studies confirmed that dietary NS is effective at reducing both DNA damage and liver cancer in AFB_1-treated trout. NovaSil, like CHL, has the potential to reduce the global impact of aflatoxin exposure on human health. Future studies will examine the effectiveness of these two agents when given in combination. LPI

Continued from page 3 — Interview with Dr. Adrian Gombart

Q. What are kinase inhibitors and what do they do?

A. We were interested in cyclin-dependent kinase inhibitors because it became clear that they would very likely be tumor suppressors. Kinases are enzymes that modify protein activity by phosphorylation, and cyclin-dependent kinases regulate the cell cycle. If you can inhibit those kinases, you can interfere with the cell cycle. Interrupting the cell cycle is strategically important in cancer therapy. When it was discovered that cyclin-dependent kinase inhibitors could block the progression of the cell cycle, they became candidates for potential tumor suppressor genes. To find out if they were tumor suppressors, we analyzed mutations in cancers from different patients. The normal cell had an unchanged copy of the gene, but the tumor suppressor gene was altered in the cancer cell because of various mutations.

Q. When did you become interested in vitamin D?

A. When I went to Cedars-Sinai Medical Center, I worked with Phillip Koeffler, a hematologist/oncologist who has worked on both leukemias and cancers involving solid tissues. His focus was on the basic biology of cancer, but he was also looking for ways to treat the disease. In the early 1980s, there was a lot of interest in using vitamin D because it can cause cell differentiation or maturation and inhibit the growth of cells. Some people were interested in analogs of vitamin D, which are compounds that have had the structure slightly changed to enhance their beneficial effects and reduce side effects. Dr. Koeffler was working on the use of vitamin D to cause leukemic cells to differentiate, which would have therapeutic value. So I got involved in that research.

Q. What does vitamin D do in the body?

A. It seems like vitamin D does everything! It's primarily known for its importance in bone growth—making strong bones and strong teeth by maintaining proper calcium levels in our blood through absorption from the gut. But it's becoming quite clear that deficiencies in vitamin D lead to a number of diseases. Vitamin D is involved in a lot of important processes in the body, including the immune system, and recent studies show the importance of vitamin D in cardiovascular health.

Q. If vitamin D is synthesized in the skin on exposure to sunlight, why is it called a vitamin?

A. A vitamin is a substance that you need to get from your diet—your body doesn't synthesize it. Vitamin D was called a vitamin because of the discovery that vitamin D-deficient animals could be fed irradiated food to cure rickets. Vitamin D is now considered a hormone because our bodies can synthesize it. Vitamin D's effects are mediated through a protein that belongs to a class of proteins called steroid hormone receptors. These transcription factors bind to certain kinds of hormones and other compounds to turn on gene expression.

Q. How many forms of vitamin D are there?

A. Vitamin D is produced from ultraviolet light hitting the 7-dehydrocholesterol molecule in the skin. The pre-vitamin D molecule travels in the blood to the liver, where it's hydroxylated and becomes 25-hydroxyvitamin D, which is the form that circulates in the blood at high levels. Physicians measure 25-hydroxyvitamin D to determine if you are deficient or sufficient. 25-Hydroxyvitamin D is then hydroxylated in the kidneys to the active compound, 1,25-dihydroxyvitamin D or calcitriol, which binds to receptors and turns on genes. While the kidneys are the primary source of biologically active vitamin D, we now know that different cell types in our bodies can produce active vitamin D. Cells of the innate immune system that kill microbes that they've engulfed can produce the active form of vitamin D.

Q. Is the vitamin D found in supplements the same form of vitamin D that's made in the body or found in food?

A. Supplements contain either vitamin D_2, also called ergocalciferol, or, usually, vitamin D_3, also known as cholecalciferol. Both are converted in the kidneys to the active form of vitamin D. Vitamin D_2 is found in plants. Vitamin D_3 is the form found in animals. Vitamin D_3 in supplements primarily comes from chemically modified lanolin from sheep wool. The forms put into supplements are chemically indistinguishable from the forms that are found naturally in food.

Q. What foods contain vitamin D?

A. Vitamin D is found in fish like salmon, herring, and sardines, and in cod liver oil. Some foods in the U.S. are fortified with vitamin D, including milk, orange juice, and some cereals, grains, and breads.

Q. Mushrooms that we find in the supermarket are typically grown in the dark. Would they contain much vitamin D?

A. They would not have much at all, but if they are flash exposed to ultraviolet light, some vitamin D is made. The longer the exposure, the more vitamin D is produced. Wild or cultivated mushrooms exposed to sunlight can have pretty high levels.

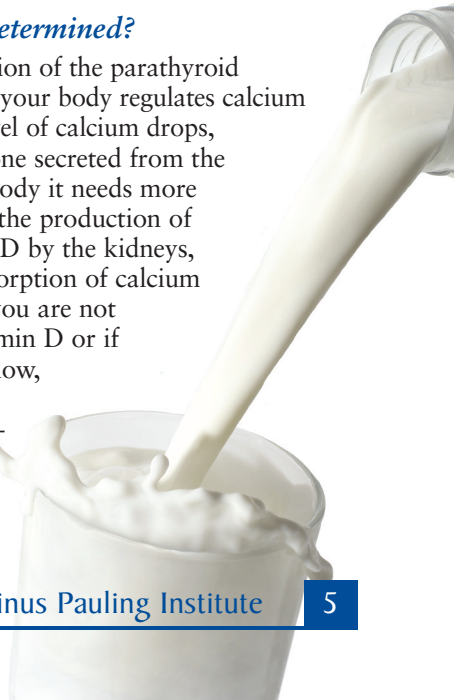
Q. How is vitamin D deficiency determined?

A. It's done by a blood test that measures 25-hydroxyvitamin D. Sufficient levels are 30-32 nanograms (ng) per milliliter of blood. From 20 to 30 ng/ml is considered insufficient. Below 20 ng/ml is deficient.

Q. How is sufficiency determined?

A. It's based on the regulation of the parathyroid hormone, which is how your body regulates calcium levels. If your blood level of calcium drops, then parathyroid hormone secreted from the parathyroid tells your body it needs more calcium. That leads to the production of 1,25-dihydroxyvitamin D by the kidneys, which increases the absorption of calcium from the intestines. If you are not consuming enough vitamin D or if levels of vitamin D are low, then you can't produce enough 1,25-dihydroxyvitamin D needed to sequester calcium.

continued on page 6



Continued from page 5 — Interview with Dr. Adrian Gombart

Q. And that explains the development of rickets?

A. Yes. Instead of getting calcium from the diet, the body takes calcium out of the bones. The parathyroid hormone is regulated by the active form of vitamin D, 1,25-dihydroxyvitamin D. If that mechanism for suppressing parathyroid hormone levels is absent, the parathyroid hormone levels increase, leading to the leeching of calcium from the skeleton. At around 30-32 ng/ml of 25-hydroxyvitamin D in your blood, the parathyroid hormone levels remain normal.

Q. It seems that good vitamin D status is important in every stage of life.

A. Yes, vitamin D levels are very important throughout life—to prevent rickets in childhood and to ensure bone health as we age. Also, it's becoming apparent that it is important for reducing the incidence of diseases that we associate with aging, including cardiovascular disease, muscle weakness, increased inflammation, and poor immune function.

Q. What is the optimum level of vitamin D in the blood, and how does supplemental vitamin D affect that?

A. It's not really known what the optimal level is, and there is a lot of research on that now. Vitamin D is involved in many physiological processes. Vitamin D status optimized for proper immune function may not be optimal for cardiovascular health or for preventing cancer. Supplements can raise your blood levels of vitamin D—every 100 IU raises blood levels by about 1 ng/ml.

Q. What factors influence the synthesis of vitamin D in the skin?

A. The synthesis of vitamin D in your skin is affected by where you live and the season. The further north you go, especially during the winter, the less vitamin D is made in the skin. Here in Oregon at around the 45th parallel, you're not going to produce much. If you live in Los Angeles, you can synthesize vitamin D year round. Sunscreen blocks vitamin D synthesis. The darker the skin, the longer you need to spend in the sunlight. As you get older, your skin becomes less efficient at synthesizing vitamin D, probably due to a reduction in the cholesterol substrate.

Q. How widespread is vitamin D deficiency?

A. It's estimated that about 10% of Americans are deficient and that 70% are insufficient.

Q. The litany of diseases that vitamin D may help prevent is long and includes cancer, heart disease, diabetes, multiple sclerosis, hypertension, autoimmune diseases like rheumatoid arthritis, and infections. How can vitamin D be effective in preventing so many different diseases?

A. One connection between those diseases is the immune system. Vitamin D is important for maintaining a balanced T lymphocyte repertoire. An imbalance could lead to increased levels of inflammation. Inflammation is important for fighting infection, but chronic inflammation probably contributes to the development of many of the diseases you mentioned. A level of vitamin D in blood of 30 ng/ml or higher may be necessary for optimal immune response, function, and control of the inflammatory response.

Q. What is the difference between innate immunity and adaptive immunity?

A. Adaptive immunity is involved in our response to vaccines and provides long-term protective immunity. T cells and B cells are important parts of adaptive immune response. B cells produce antibodies in response to antigens from pathogens like bacteria or viruses. T cells release toxins to kill pathogens. The innate immune system responds quickly to pathogens in a non-specific manner. Cells of the innate immune system like macrophages and neutrophils engulf pathogens and kill them but don't retain a memory of those pathogens.

Q. How does vitamin D help kill pathogens?

A. We discovered that vitamin D turns on genes in macrophages and neutrophils. Those genes make small peptides called cathelicidin that punch holes in the pathogen's membrane and disable it.

Q. Is vitamin D absolutely required for the cathelicidin peptide to be made?

A. Yes. The model that seems to be developing is that activation of toll-like receptors—proteins that recognize microbial molecules—cause macrophages to ramp up production of vitamin D and its receptor, leading to the production of cathelicidin. Much of the early cell culture work in this area was inconsistent, probably because there wasn't much vitamin D present in the culture media. Another group at UCLA led by Robert Modlin showed that the antimicrobial response was dependent on vitamin D in cultured cells. Their work indicated that low vitamin D levels in the blood would not support the production of this important antimicrobial peptide by macrophages.

Q. How important is cathelicidin among these antimicrobial peptides?

A. It seems to be quite important. There are a lot of defenses that work together to battle pathogens, but cathelicidin is critical. There's a knock-out mouse model in which cathelicidin isn't present, and the mice are susceptible to skin, eye, and urinary tract infections, and their gut is colonized by bacteria much more easily.

Q. Is cathelicidin found in most animals?

A. In humans and closely related primates there is only one cathelicidin gene that is regulated by vitamin D. In animals, vitamin D is important for immune response but not as a regulator of cathelicidin.

Q. How was the discovery of the relationship between vitamin D and the cathelidicin antimicrobial peptide made?

A. It was made when I was working with Phil Koeffler at Cedars-Sinai Medical Center. We were working on the differentiation of myeloid cells to become innate immune cells called macrophages and neutrophils. I was studying diseases where there were defects in that process and looking for ways to turn on the antimicrobial peptide genes with compounds that might provide a potential treatment for disease. I discovered that the active form

of vitamin D strongly induced cathelicidin in the macrophage-like cells that we were studying. As we were getting ready for publication, another paper came out that reported the observation that I made—that there are vitamin D-binding sites in the cathelicidin promoter. We had a grant submitted and a patent application filed, and our paper was published a few months later. Furthermore, we reported that vitamin D enhances cathelicidin production in numerous cell types and in macrophages, and reported that the cathelicidin gene has been evolutionarily conserved in primates as an important part of innate immunity. We followed that up with a paper last year showing that the expression of cathelicidin gene is controlled by so-called "junk DNA" and has been conserved in the human lineage for 55 to 60 million years.

Q. ***Have there been any epidemiological studies that looked at blood levels of vitamin D and rates of infection or incidence of disease?***

A. There are a lot of anecdotal reports, but most of the published studies deal with mycobacteria or using vitamin D to treat tuberculosis. Before antibiotics were developed, TB patients exposed to sunlight in sanitariums seemed to improve. No one knew what was responsible for that therapeutic effect. There is a lot of evidence showing that deficiency of vitamin D is associated with reactivation of the disease. For example, in TB patients from Southeast Asia who move north, the disease gets reactivated because they get less sunlight and tend to be vegetarian and covered more in clothing. A recent report analyzing data from the NHANES epidemiological study showed a correlation between vitamin D deficiency and increased respiratory tract infections.

Q. ***Has there been any interest in combining vitamin D supplementation with drug therapy for tuberculosis?***

A. There are a number of ongoing trials. One trial was published but wasn't very promising, probably because of the way it was designed. It will be interesting to see the results from the ongoing trials.

Q. ***Your study in patients undergoing chronic hemodialysis showed that those with the lowest blood level of cathelicidin had an increased risk of death from infection. How big was the effect and have there been any follow-up studies using vitamin D supplementation to try to raise cathelicidin levels in the blood?***

A. Cathelicidin is secreted into the blood and is present at pretty high levels. Most antimicrobial peptides are packaged in neutrophils, which are white blood cells, but cathelicidin is also actively secreted into the blood for some unknown purpose. There isn't any research that correlates vitamin D status with cathelicidin levels in blood. Most of our patients had pretty high levels of vitamin D, so we really need to look at a group of people who have marginal or deficient levels of vitamin D. In the study with dialysis patients, vitamin D compounds were used to suppress parathyroid hormone levels. Some studies have found that hemodialysis patients who take vitamin D have a sharply reduced risk for cardiovascular

mortality. In our dialysis study, we measured vitamin D and cathelicidin in the blood of several hundred patients in a prospective cohort of over 10,000 patients undergoing hemodialysis. Those with the lowest level of cathelicidin had a two-fold increased risk of death from infection within the first year of hemodialysis compared to those with the highest levels of cathelicidin. We found a positive association between the active form of vitamin D—1,25-dihydroxyvitamin D—and cathelicidin levels. We are following this up with a clinical study at Cedars-Sinai Medical Center with sepsis patients.

Q. ***Hospital infections are a great cause for concern because of increased morbidity and mortality. One recent study found that the risk for morbidity, organ failure, and length of stay in the ICU among surgical trauma patients was substantially reduced by supplemental vitamins C and E given prior to surgery. Do you think that vitamin D should also be tried in that context?***

A. I think it's worth considering. A lot of people going into the hospital have insufficient or deficient levels of vitamin D and are more likely to develop sepsis. The very elderly are even more likely to be deficient in vitamin D. Multivitamin supplements are usually given to patients in the hospital, but I think they could really consider higher doses of vitamin D to boost immunity.

Q. ***You mentioned the effect of vitamin D on vascular disease in dialysis patients. How is that explained?***

A. It's not well understood, but it may involve the relationship between vitamin D and parathyroid hormone levels or the effects of vitamin D on blood pressure, since adequate vitamin D status decreases the risk for high blood pressure. Vitamin D has anti-inflammatory properties. Chronic inflammation increases the development of cardiovascular disease and keeping it in check could prove beneficial.

Q. ***We've talked about vitamin D and bone health and immunity. Are there other roles for vitamin D in older people?***

A. It's becoming apparent that vitamin D is important for preventing falls due to its role in maintaining muscle strength. That's especially important in older adults. Vitamin D seems to help prevent muscle atrophy and is important in the calcium regulation of muscle activity. There are also intriguing correlative studies suggesting that vitamin D may improve cognitive function in the elderly.

continued on page 8

Continued from page 7 — Interview with Dr. Adrian Gombart

Q. Why have clinicians been interested in the treatment of breast cancer and prostate cancer with vitamin D?

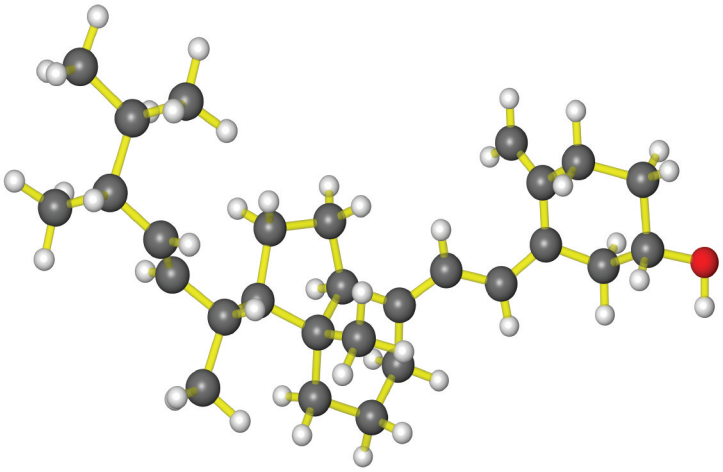
A. Much preclinical data show that active metabolites of vitamin D prevent the growth of those cancer cells, probably by inducing the tumor suppressor genes and also by causing the cells to differentiate and stop proliferating. The active metabolites have been shown to increase the efficacy of chemotherapeutic agents in preclinical models. Also, high circulating serum levels of vitamin D correlate with lower rates of numerous cancers.

Q. The expert consensus on the recommended daily intake of vitamin D seems to be changing. For example, pediatricians recently recommended higher intakes for infants and children. Do you think that the Food and Nutrition Board of the Institute of Medicine will increase the dietary recommended intakes of vitamin D for adults?

A. They are considering that right now. I think that there will be an increase because there is a lot of evidence to support it.

Q. Does taking supplemental vitamin D seem sensible to you?

A. LPI recommends 2,000 IU per day, and I agree with that. I take about 2,500 IU per day. It is important to keep sufficient vitamin D levels in the blood. It can be taken daily all year, and it's better to keep levels consistently high rather than have them go up and down. And there's no problem with any toxicity at these doses. You'd have to take 100,000 IU or more per day long term to cause toxicity. Our bodies are capable of synthesizing about 20,000 IU/day through sun exposure! **LPI**



Vitamin E and Chemotherapy

Debbie J. Mustacich, Ph.D.
Assistant Professor (Senior Research)
Linus Pauling Institute and OSU
Department of Biomedical Sciences

The American Cancer Society estimates that more than 1.4 million new cases of cancer were diagnosed and more than 500,000 people died of cancer-related deaths in the United States during 2008. Lung cancer accounts for 29% of all cancer deaths and is the leading cause of cancer deaths in both men and women. Non-small-cell lung cancer (NSCLC) accounts for 75-80% of lung cancers and has a five-year survival rate of ~15%. In women, ovarian cancer has the highest mortality rate of all the female reproductive system cancers, accounting for 6% of all cancer-related deaths in women. The high death rate for both of these cancers is due, in most cases, to patients having advanced disease at the time of diagnosis. In contrast, prostate cancer is often diagnosed in the early stages when the tumors are responsive to androgen ablation therapy. However, over time prostate cancers nearly always undergo androgen-independent progression and become refractory to hormonal manipulation such that prostate cancer remains the second leading cause of cancer-related deaths in men. These dismal statistics indicate a need for innovative improvements in treatment modalities, particularly with respect to improved therapies for patients with advanced stage cancer.

Why use cisplatin?

Cisplatin, a platinum-containing compound, is currently one of the most effective anticancer drugs available for treating a variety of solid tumors, including lung, ovarian and prostate cancer. Cisplatin-based chemotherapy has been shown to significantly increase overall survival rates when used as adjuvant treatment in NSCLC patients following lung resection surgery, particularly in late-stage cancer. In addition, clinical trials have demonstrated an increased response and median survival in hormone refractory prostate cancer (HRPC) patients treated with therapies that include platinum compounds. In 2006, the National Cancer Institute recommended that the standard treatment for women with late-stage ovarian cancer should be surgical removal of the bulk of the tumor followed by intraperitoneal (IP) injection of cisplatin in combination with a taxane, such as paclitaxol, given either IP or intravenously (IV). Use of IP cisplatin in ovarian cancer patients increased median survival by 16 months compared to IV cisplatin. Compared to IV administration, IP administration of chemotherapy drugs allows longer exposure of the peritoneal cavity, the principal site of disease in ovarian cancer, to higher doses of the drug. Unfortunately, like many other anticancer agents, platinum compounds are neurotoxic, and development of peripheral sensory neuropathy often limits the dose and duration of

treatment. Notably, neurologic side effects occurred at a higher rate in patients receiving IP cisplatin such that most patients receiving IP cisplatin did not complete as many treatment cycles as patients receiving IV cisplatin. The mechanism of cisplatin neurotoxicity remains unknown, thus hampering the development of optimal adjuvant treatments to prevent platinum-induced neuropathy and allow patients the full life-extending benefits of cisplatin treatment without the debilitating side effects that reduce quality of life.

Similarities between cisplatin toxicity and vitamin E deficiency neuropathies

Vitamin E deficiency occurs rarely in humans. When it does, it is usually the result of a genetic abnormality in the alpha-tocopherol transfer protein or a fat malabsorption syndrome. A distinct pattern in the progression of neurological symptoms resulting from vitamin E deficiency in humans has been described, with symptoms characterized by ataxia (loss of muscle coordination), paresthesia (prickling, tingling sensation), and loss of reflexes. These symptoms result from a loss of the large caliber axons of the sensory neurons. Pathologic studies indicate that the dorsal root ganglia are the neural tissue primarily affected by vitamin E deficiency. Remarkably, the description of neuropathy in cancer patients treated with cisplatin closely resembles that of human vitamin E deficiency. In one study, 10 of 11 ovarian cancer patients receiving cisplatin developed a distal sensory neuropathy, manifested by decreased vibratory sensation—the first detectable symptom in vitamin E deficiency. With continued cisplatin therapy, four patients developed paresthesia. At autopsy, platinum levels were highest in the dorsal root ganglia, as compared to other neural tissue, the same nervous tissue affected by vitamin E deficiency.

Benefits of vitamin E supplementation during cisplatin treatment

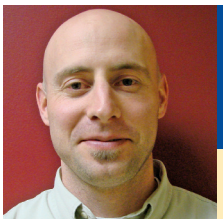
Many anticancer drugs cause oxidative stress in normal tissues while killing cancer cells via non-oxidative stress mechanisms. Importantly, cisplatin-based regimens have been shown to decrease plasma vitamin E (alpha-tocopherol) levels in patients. Thus, antioxidants, including vitamin E, may represent an important adjuvant to standard chemotherapy regimens. However, clinical trials investigating the use of alpha-tocopherol in combination with chemotherapeutic drugs have been sparse, in part due to the concern that antioxidant vitamins, due to their ability to prevent oxidative stress generated by free radicals, may reduce the efficacy of some anticancer drugs. However, numerous cell culture studies have found that vitamin E, particularly at high pharmacologic doses, potentiates the apoptotic (programmed cell death) and growth inhibitory effects of anticancer drugs, including platinum compounds.

Ongoing studies

Our central hypothesis is that 1) cisplatin depletes alpha-tocopherol in the lumbar region of the spinal cord, specifically the dorsal root ganglion, by an oxidative stress mechanism whereby platinum acts as a catalyst for lipid peroxidation resulting in an alpha-tocopherol-deficiency neuropathy; 2) pharmacologic doses of alpha-tocopherol will prevent the cisplatin-mediated alpha-tocopherol depletion, thereby preventing the neurologic side effects of cisplatin; and 3) alpha-tocopherol supplementation will not decrease the anticancer efficacy of cisplatin.

We first determined that daily administration of pharmacologic doses of alpha-tocopherol to rats significantly increased alpha-tocopherol levels in tissues, including liver (~15-fold), lung (~3-fold), kidney (~2-fold), spinal cord (~2-fold), and dorsal root ganglia (~2-fold). Next, we treated rats with three cycles of five daily injections of either cisplatin or placebo with and without co-administration of pharmacologic doses of alpha-tocopherol. As expected, lipid peroxidation significantly increased and dorsal root ganglia alpha-tocopherol levels significantly decreased in cisplatin-treated rats. Importantly, concurrent high-dose alpha-tocopherol administration preserved dorsal root ganglia alpha-tocopherol levels and prevented increased lipid peroxidation in cisplatin-treated rats. Future studies to further elucidate the mechanism of cisplatin-induced neuropathy, as well as determine the efficacy of adjuvant high-dose alpha-tocopherol, will determine 1) the ability of cisplatin to alter the plasma and tissue levels of additional antioxidants; 2) the ability of alpha-tocopherol to prevent the morphological changes in dorsal root ganglia associated with cisplatin-induced neuropathy; and 3) the anticancer efficacy of cisplatin in tumor-bearing rats given pharmacologic doses of alpha-tocopherol.

Understanding the mechanism of cisplatin-induced neuropathy, as well as determining the ability of alpha-tocopherol to prevent the biochemical and morphologic changes associated with cisplatin-induced neuropathy without decreasing anticancer efficacy, will provide the necessary evidence-based knowledge to support clinical trials to determine guidelines for including vitamin E in cisplatin-based chemotherapy regimens. Preventing cisplatin-induced neuropathies will allow patients to complete, and possibly increase, their cisplatin treatments; thus allowing patients to fully benefit from the life-extending potential of long-term cisplatin treatment without the debilitating side effects that currently reduce quality of life for both patient and family. LPI



Vitamins E and C and the Recovery from Anterior Cruciate Ligament Surgery

Tyler Barker, LPI Graduate Fellow

The anterior cruciate ligament (ACL) is an integral component of knee structure and function. Its role is to limit excessive knee rotation and provide stability to the knee joint. Unfortunately, every year approximately 200,000 Americans injure their ACL. In nearly half the cases, this injury to the ligament requires reconstructive surgery. Immediate and persistent muscle dysfunction commonly follows the injury and surgical repair of a ruptured ACL. Despite advances in orthopedic surgery, physical therapy, and medicine, muscle dysfunction, especially weakness, can continue for years. Although impaired neurological feedback from the surgically repaired knee and reduced physical activity contribute to the muscle dysfunction, the reasons for muscle atrophy and weakness remain a mystery.

We are interested in ACL injury because oxidative stress may play an important role in exacerbating muscle degeneration. Both oxidative stress and pro-inflammatory molecules called cytokines induce muscle dysfunction during aging and in chronic heart failure patients. Therefore, our findings may be applicable to people other than those with ACL injury. Because vitamins E and C are potent dietary antioxidants that modulate diverse inflammatory cytokines, we conducted a randomized, double-blind, placebo-controlled study to investigate the influence of vitamin E and C supplementation on the recovery from ACL reconstructive surgery. We made three major discoveries that we discuss further below.

1. Vitamin E and C supplementation lowers the increase of an anti-inflammatory cytokine (interleukin-10 or IL-10) and stabilizes the depression of a pro-to-anti-inflammatory cytokine ratio (i.e., IL-6:IL-10) immediately following surgery.
2. The recovery of leg strength following ACL reconstruction is associated with vitamin C levels in blood prior to surgery and with elevated vitamin C levels post-surgery following vitamin E and C supplementation.
3. Greater strength gains after ACL surgery are associated with lower oxidative stress and a higher pro-to-anti-inflammatory cytokine ratio but only in the subjects supplemented with vitamins E and C.

Vitamin E and C supplementation improves inflammatory status immediately following ACL surgery

During ACL surgery, a tourniquet is applied to the upper thigh of the leg that is being repaired. This procedure provides a bloodless operating field but also creates an ischemic condition in which blood and oxygen are deprived from the affected area. When the tourniquet is removed and blood begins to circulate again, the tissue is subject to what's known as ischemia-reperfusion injury. The restored flow of blood causes oxidative and inflammatory damage. Ischemia-reperfusion injury is potentiated by oxidative stress, which stimulates pro-inflammatory cytokines that further exacerbate the inflammation. Although it has been documented that there is an increase in local markers of oxidative stress following tourniquet removal, the role of the circulating inflammatory cytokine and oxidative stress marker responses to this ischemia-reperfusion insult has not been well described.

Therefore, we examined oxidative stress and inflammatory cytokines in the circulation after ACL surgery in subjects who received either a placebo or an antioxidant supplement of vitamins E (400 IU) and C (1,000 mg), taken in two equally divided daily doses starting approximately two weeks prior to surgery and continuing for three months after surgery.

As expected, we found that antioxidant supplementation doubled the vitamin E and C plasma concentrations. Nonetheless, within 90 minutes following ACL surgery, we found a significant elevation in oxidative stress in both the supplemented and placebo groups. There were no significant differences in markers of lipid peroxidation—F_2-isoprostanes or malondialdehyde—between the groups. These markers decreased to baseline levels a few days following surgery. Additionally, there were increases in markers of muscle damage (creatine kinase) three days after surgery. Inflammation was also increased at three and seven days following surgery, as indicated by an elevation in the pro-inflammatory cytokine IL-6 and C-reactive protein. However, antioxidant supplementation did not affect biomarkers of muscle damage and inflammation following surgery.

An immune response typically follows surgery, represented by elevated blood levels of the anti-inflammatory cytokine IL-10. At 90 minutes following ACL surgery, IL-10 increased markedly in the placebo but not the antioxidant group. This is remarkable because it suggests that the antioxidant supplementation decreased the body's need to express an anti-inflammatory cytokine. Vitamins E and C potentially prevented an increase in oxidative stress and/or pro-inflammatory cytokines and provided protection against post-surgery complications. However, further research addressing this question is required.

Another approach to assessing inflammation is to calculate ratios between pro-inflammatory (IL-6) and anti-inflammatory (IL-10) cytokines. Changes in the IL-6:IL-10 ratio, for example, may have prognostic value for poor clinical outcomes, such as infection or death. We observed that the IL-6:IL-10 ratio decreased immediately following ACL surgery. However, this depression was ameliorated in the subjects who received vitamins E and C. Whether the stabilized pro-to-anti-inflammatory cytokine ratio in our antioxidant group translates to improved recovery following surgery is unknown but warrants further investigation.

Greater strength gains after ACL surgery are associated with lower oxidative stress only in subjects supplemented with vitamins E and C

It is unknown if oxidative stress or inflammatory cytokines are associated with leg weakness following ACL surgery and whether supplementation with vitamins E and C would modulate this relationship. We found that the maximum isometric force produced by the injured leg following ACL surgery was inversely correlated with plasma F_2-isoprostanes—markers of oxidative stress—within the antioxidant group. That is, the less oxidative stress, the stronger the leg. Furthermore, maximum isometric force of the injured leg also correlated with the IL-6:IL-10 cytokine ratio in the antioxidant group. Therefore, our results indicate that antioxidants decrease oxidative stress, improve the balance of pro- and anti-inflammatory cytokines, and improve the recovery from muscle damage.

Better recovery of leg strength following ACL reconstruction is associated with high plasma vitamin C levels

High levels of vitamins E and C are associated with improved strength and physical performance in the elderly. Whether supplemental vitamins E and C can improve limb strength is unknown. Therefore, we measured plasma vitamin E and C concentrations before and three months after ACL surgery in our two experimental groups described above. Not surprisingly, the injured leg was weaker than the other leg at baseline, and it remained so three months after surgery. However, greater gains in leg strength after surgery were associated with higher plasma vitamin C levels prior to surgery and with elevated vitamin C levels following supplementation, suggesting that vitamin C aids muscle recovery from ACL surgery.

Our data indicate that supplemental vitamins E and C provided protection against undesirable immunological activity after surgery. The reduction of the anti-inflammatory cytokine IL-10 by antioxidant supplementation following surgery may have provided protection against complications, as seen in other conditions. Interestingly, in the subjects supplemented with vitamins E and C, lower oxidative stress and a higher pro-to-anti-inflammatory cytokine ratio were associated with greater leg strength three months after ACL reconstruction. In conclusion, our results suggest that there is a potential therapeutic benefit of vitamin E and C supplementation on both immediate surgically-induced immunological changes and with the short-term recovery of leg strength following ACL reconstruction. Antioxidant supplementation may be of therapeutic benefit to patients suffering from other conditions involving oxidative stress and inflammation. LPI

Do "Antioxidants Prevent Health-Promoting Effects of Physical Exercise in Humans"?

Scott W. Leonard, LPI Senior Research Assistant

A paper published in 2009 by Ristow *et al.* in the *Proceedings of the National Academy of Sciences*, "Antioxidants Prevent Health-Promoting Effects of Physical Exercise in Humans", cast doubt on the combined health benefits of antioxidant consumption and exercise. While the data do not appear to be flawed and are important, we believe the authors have overstated their findings.

There is no doubt that achieving and maintaining a healthful weight is a benefit of exercise, but the molecular mechanisms underlying this effect are unclear. Ristow proposed that reactive oxygen species (ROS) have an essential role in promoting insulin sensitivity during exercise, one of the known benefits of exercise. They then claimed that antioxidant use, leading to a decrease in ROS, is detrimental to the health-promoting effects of exercise. There are many good studies, including several from our labs, showing benefits of antioxidant use during exercise in decreasing markers of oxidative stress. Antioxidants have been shown to decrease DNA damage, lipid peroxidation, and protein oxidation following exercise. The combination of antioxidants and exercise has also been shown to delay brain aging, a phenomenon proposed to be related to oxidative stress. Ristow reported that antioxidant supplementation blocked the induction of antioxidant enzymes in the body. However, the supplemental antioxidants may have negated the need for up-regulation of endogenous antioxidant proteins like superoxide dismutase and glutathione-synthesizing enzymes.

The study's authors focused on changes in insulin sensitivity, but it is known that contracting skeletal muscle can take up glucose independently of insulin during exercise, and glucose uptake is normal during exercise in diabetics, who were excluded from the study. The authors tried to link their findings to the molecular changes that occur in type 2 diabetics, but their choice of healthy men instead of diabetics made that problematic. It is well accepted that there are health benefits from exercise and antioxidant consumption, whether from the diet or supplementation, alone or in combination. Indeed, a diet rich in fruits and vegetables and regular exercise have long been recognized to decrease the risk for many chronic diseases.

The statement by Ristow that "fruits and vegetables may exert health-promoting effects despite their antioxidant content" seems particularly inappropriate. Humans *depend* on fruits and vegetables and other dietary sources for the important physiological antioxidants, vitamins C and E. In a recent commentary on the oxidative stress paradigm discussed at the third international symposium on "Nutrition, oxygen biology and medicine-micronutrients, exercise, energy and aging disorders" in Paris, Dr. Regina Brigelius-Flohé commented that "it remained obvious that a lot of work is needed to fully understand the conditions and individual situations under which ROS are beneficial or detrimental". LPI

Nutrition and Immunity, Part I

Victoria J. Drake, Ph.D.
LPI Research Associate

The immune system functions to protect the body against infection and disease. It is a complex and integrated network of cells and organs that defends against pathogenic organisms and guards against the development of cancer. The immune system consists of the innate immune system and the adaptive immune system. This article explores the role of macronutrients and vitamins on immune function. An article in a forthcoming newsletter will address the role of minerals and other factors in immunity.

The innate immune system is an immediate, nonspecific response to harmful substances. It is composed of physical barriers, such as the skin, as well as chemical and microbiological barriers, including the mucous secretions of the respiratory tract and the normal microflora of the gastrointestinal tract. Primary components of the innate immune system include monocytes, macrophages, and neutrophils—immune cells that engulf and digest invading microorganisms in a process called phagocytosis. These cells express surface receptors that identify pattern-recognition molecules that are conserved among several families of pathogens but unique to pathogenic microorganisms. A key component of innate immunity is the complement system, a biochemical cascade of at least 20 serum proteins that functions to kill invading pathogens by direct lysis (cell rupture) or through the promotion of phagocytosis. Innate immunity also involves cells that mediate the inflammatory response. Thus, the innate immune response is a rapid, nonspecific response that serves as the first line of defense against invading microorganisms. However, if the innate response doesn't adequately eliminate microorganisms and resultant infections, the adaptive immune system is summoned to action through the signaling of messenger proteins called cytokines.

The adaptive or acquired immune system is a second line of defense against pathogens. This defense develops over a longer period of time, taking several days to even weeks to develop. Compared to the innate response, adaptive immunity is much more complex because it involves antigen-specific responses and immunologic "memory" of pathogenic organisms. Exposure to a specific antigen on an invading pathogen stimulates production of immune cells that target the pathogen for destruction; subsequent responses to the same pathogen are stronger and more efficient because the antigen is "remembered." The major mediators of the adaptive immune response are specialized white blood cells, namely B lymphocytes (B cells) and T lymphocytes (T cells). B cells produce antibodies and are responsible for humoral immunity, while T cells are responsible for cell-mediated immunity. Although the innate and adaptive immune systems have unique functions, their components interact and work together to protect the body from infection and disease.

The ability of the immune system to prevent infection and disease is strongly influenced by nutritional status of the host. In fact, malnutrition is the most common cause of immunodeficiency in the world. Poor overall nutrition can lead to inadequate intake of energy and macronutrients as well as selected micronutrient deficiencies. These nutrient deficiencies can cause immunosuppression and dysregulation of immune responses. Specifically, nutritional deficiencies can impair phagocyte function in innate immunity and cytokine production in adaptive immunity, as well as adversely affect certain aspects of humoral and cell-mediated immunity. Impairment of these responses can compromise the integrity of the immune system, thereby increasing one's susceptibility to infection. Because nutritional status can modulate the actions of the immune system, the sciences of nutrition and immunology are tightly linked.

Inadequate intake of macronutrients or selected micronutrients can lead to immune deficiency, impaired host-defense mechanisms, and therefore, increased susceptibility to infection and disease. Protein-energy malnutrition (PEM), also called protein-calorie malnutrition, is a common nutritional problem worldwide. Primary PEM, which is caused by insufficient intake of protein and/or energy, is more common in developing nations but is also present in certain subgroups in industrialized nations, such as the elderly and individuals who are hospitalized. Secondary PEM is more common in developed countries, often occurring in the context of a chronic disease that interferes with nutrient metabolism, such as inflammatory bowel disease, chronic renal failure, or cancer. Regardless of the specific cause, PEM primarily affects cell-mediated immunity rather than humoral immunity. In particular, PEM leads to atrophy of the thymus, the organ that produces T cells, which reduces the number of circulating T cells and decreases the effectiveness of the memory response to antigens. Humoral immunity is affected to a lesser extent, but antibody affinity and response is generally decreased in PEM. PEM also leads to decreased production of certain cytokines, reduced levels of several complement proteins, and phagocyte dysfunction. Additionally, PEM compromises the integrity of mucosal barriers, thereby increasing susceptibility to infections of the respiratory, gastrointestinal, and urinary tracts. PEM often occurs in combination with deficiencies in essential micronutrients, especially vitamin A, zinc, copper, selenium, and magnesium.

Several types of dietary lipids (fatty acids) have been shown to modulate immune function. Polyunsaturated fatty acids (PUFAs)—fatty acids with more than one double bond between carbons—are broadly divided into two main classes: omega-3 PUFAs and omega-6 PUFAs. Lipids of both classes are essential nutrients because they cannot be synthesized by the body; therefore, PUFAs must be obtained from the diet. Dietary sources of omega-3 PUFAs include oily fish, flaxseeds and their oil, walnuts and their oil, and canola oil. Food sources of omega-6 PUFAs include vegetable oils (e.g., soybean, safflower, and corn oil), nuts, and seeds.

Eicosanoids (20-carbon PUFA derivatives) are signaling molecules that play important roles in inflammatory and other immune responses. In general, eicosanoids derived from omega-3 PUFAs have anti-inflammatory and

immunosuppressive effects, while eicosanoids derived from omega-6 PUFAs tend to have mostly pro-inflammatory and immunostimulatory effects, although some omega-6 PUFA derivatives (i.e., lipoxins) have anti-inflammatory properties. While high intakes of omega-3 PUFAs may benefit individuals with inflammatory or autoimmune diseases, increased omega-3 PUFA intakes, especially at supplemental doses, could impair host-defense mechanisms and increase vulnerability to infectious disease. Additionally, conjugated linoleic acid (CLA), a class of isomers of linoleic acid (an omega-6 fatty acid), has been shown in one human study to increase plasma levels of immunoglobulin antibodies IgA and IgM, decrease pro-inflammatory cytokines, and increase an anti-inflammatory cytokine. CLA is found naturally in meat and milk of ruminants but is also available in supplemental form. However, more research is needed to confirm the immune effects of CLA in humans.

In addition to macronutrients, deficiencies in certain micronutrients (vitamins and nutritionally-essential minerals) adversely affect both the innate and adaptive immune systems. Micronutrients play crucial roles in the development and expression of the immune response, and certain micronutrient deficiencies lead to immunosuppression and increased susceptibility to infection and disease. Select micronutrient deficiencies are common, especially in cigarette smokers, alcoholics, pregnant and lactating women, and the elderly. Micronutrient deficiencies often occur in the context of general malnutrition; however, common isolated micronutrient deficiencies include vitamin A, vitamin D, zinc, and iron.

Vitamin A and its metabolites are required for normal functioning of the immune system. The skin and mucosal cells (cells that line the airways, digestive tract, and urinary tract) function as a barrier and form the body's first line of defense against infection. Vitamin A maintains the integrity and function of these cells; therefore, deficiency in this micronutrient results in loss of integrity of the mucosal barriers and increased vulnerability to respiratory and diarrheal diseases and other infections. The vitamin A derivative, retinoic acid, functions as a hormone to regulate more than 500 genes, several of which are involved in controlling the production, proliferation, and differentiation of immune cells, including neutrophils, monocytes, and lymphocytes. Thus, vitamin A deficiency compromises the function of cell-mediated immunity and also impairs antibody responses to antigens, adversely affecting humoral immunity. Additionally, vitamin A deficiency adversely affects components of innate immunity, such as phagocyte function and the activity of so-called natural killer cells, and alters cytokine signals that influence various immune responses. Vitamin A deficiency is common worldwide, especially in developing nations, and has detrimental effects on maternal and child health. Severe vitamin A deficiency in children increases morbidity and mortality related to measles, malaria, and diarrheal infections; mild vitamin A deficiency may increase rates of certain infections, as well. Vitamin A supplementation can enhance immunity, decrease susceptibility to infection, and significantly reduce infection-related morbidity and mortality in children.

The active form of vitamin D—1,25-dihydroxyvitamin D—is a potent modulator of the immune system. Within a cell's nucleus, 1,25-dihydroxyvitamin D associates with the vitamin D receptor (VDR), a transcription factor that regulates gene expression. The VDR is expressed in most cells of the immune system; thus, vitamin D status can profoundly impact immunity. Vitamin D can affect aspects of both innate and adaptive immunity, including phagocytosis, cytokine production, lymphocyte differentiation, and antibody production. Additionally, the active form of vitamin D stimulates the expression of antimicrobial peptides, which are synthesized by various immune cells and function as critical components of the innate immune system. Vitamin D deficiency has been linked to an increased risk of autoimmune diseases (e.g., type 1 diabetes, multiple sclerosis, and rheumatoid arthritis) and certain cancers (colorectal, breast, and prostate). For more information about vitamin D and immunity, see the interview with Dr. Adrian Gombart in this newsletter.

Other vitamins play critical roles in immunity, such as the antioxidant vitamins C and E. Vitamin C is a highly effective antioxidant that protects the body's cells against reactive oxygen species that are generated by immune cells to kill pathogens. Immune cells accumulate vitamin C in high concentrations, which protects them against oxidative damage. Some studies have shown that vitamin C stimulates the production and function of neutrophils, lymphocytes, and phagocytes. Cell culture studies have demonstrated a direct antiviral effect of vitamin C, but clinical studies have had mixed results. Vitamin C also regenerates vitamin E, a lipid-soluble antioxidant that protects the integrity of cell membranes, from its oxidized form. Vitamin E supplementation in the elderly has been shown to improve age-related declines in immune function, particularly aspects of cell-mediated immunity. Some studies have found that this translates to an increased resistance against infections. In addition to the antioxidant vitamins, several B vitamins, such as vitamin B_6, folate, and vitamin B_{12}, are important in immunity. Vitamin B_6 deficiency impairs aspects of both humoral and cell-mediated immunity due to its requirement in the biosynthesis and metabolism of amino acids—the building blocks of proteins like cytokines and antibodies. Deficiencies in either folate or vitamin B_{12} can also adversely affect immune responses because these B vitamins are needed in the biochemical reactions that synthesize the nucleic acids DNA and RNA.

Poor overall nutrition can lead to nutritional deficiencies that compromise immunity and increase susceptibility to infection and disease. Even subclinical deficiencies in various nutrients may have adverse effects on the immune system. Infection and illness can, in turn, exacerbate states of malnutrition in many different ways, for example, by reducing nutrient intake, impairing nutrient absorption, increasing nutrient losses, or altering the body's metabolism such that nutrient requirements are increased. Immune function also declines with age. For example, older people often exhibit decreased vitamin D synthesis in the skin and may have impaired vitamin C uptake. States of malnutrition and infection can aggravate each other and lead to a vicious cycle. Eating a healthful diet and taking a daily multivitamin-mineral supplement as nutritional insurance will help to maintain optimal immune function. Single nutrient supplements, such as vitamin D, may also be necessary. LPI

Epigenetics

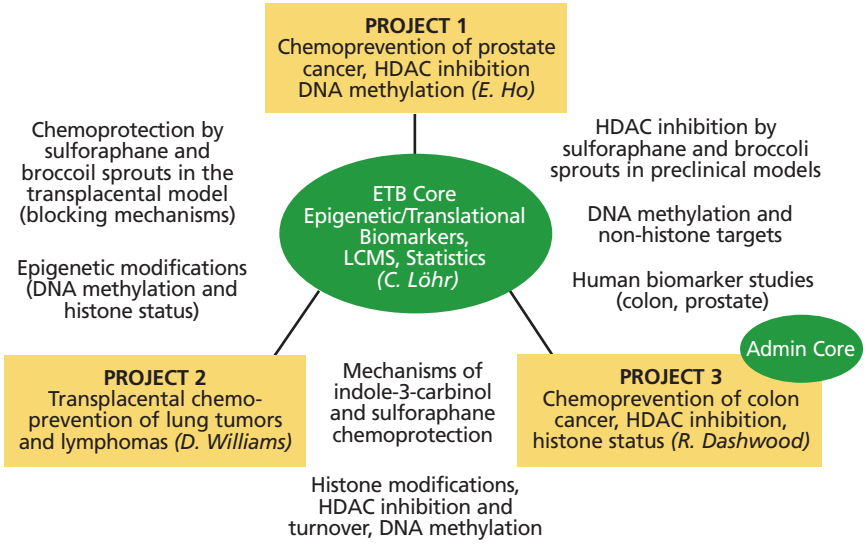
Rod Dashwood, Ph.D., LPI Principal Investigator
Director, LPI Cancer Chemoprotection Program

"Epigenetics" has finally arrived front-and-center on the popular landscape. A recent cover of *Time* magazine showed an image of double-stranded DNA being unzipped next to the words, "Why your DNA isn't your destiny". The cover stated: "The new science of epigenetics reveals how the choices you make can change your genes – and those of your kids". Just how "new" this science really is can be debated, since it likely goes back to Darwin and Lamarck and opposing views of nature *versus* nurture. Whereas Darwin argued that incremental changes underlie the process of natural selection and survival-of-the-fittest, Lamarck postulated that some traits were acquired within a lifetime due to environmental pressures. The *Time* article cited the example of Norrbotten county, Sweden. This cold and desolate area historically has been associated with periods of feast-and-famine, and research has shown that boys who went from normal eating to gluttony in a single season produced sons and grandsons who lived shorter lives. Another well-known example is the agouti mouse, which is fat, has a yellow coat, and is prone to cancer and diabetes. If the diet given to female agouti mice before conception is changed by adding chemicals called methyl donors (e.g., folate), the offspring have brown coats and are slim, despite having the same agouti gene sequence. Offspring of the mice fed their usual diet look like their parents. How might such rapid changes occur, and in a trans-generational manner? The answer seems to lie within the realm of epigenetics.

Epigenetics is the science that seeks to explain how changes in gene expression can occur without changes in the underlying DNA sequence. For example, a central theme in the cancer research field has been that mutations or large-scale chromosome rearrangements can alter the DNA sequence of key genes that regulate the fate of cells. If a gene normally expresses a protein that acts as a "brake", and this gene is mutated, then the corresponding dysfunctional protein no longer blocks unrestrained cell growth, which is a hallmark of cancer. Such tumor suppressor genes, however, also may have a normal DNA sequence, and yet the gene is aberrantly silenced in cancer. One way this can occur is by the addition to DNA of methyl groups. If these methyl groups accumulate on the 'start' region of the gene, the complex cellular machinery needed to turn on that gene is disrupted. The cellular machinery also can be blocked from even accessing the DNA in the first place. DNA is not naked in a cell but is surrounded by proteins called histones, which dynamically open and close to permit access to DNA at the right time and place. In cancers, over-expression of an enzyme called histone deacetylase (HDAC) leads to acetyl groups being removed from histone proteins, tightening their interactions with the DNA, and thereby switching off tumor suppressor genes. In essence, the "brake" is turned off, and cells continue to replicate without restraint.

Drugs that inhibit HDAC have shown promise in the clinic as therapeutic agents. They cause aberrantly silenced genes to be turned back on, thereby triggering cancer cells to commit suicide, also known as apoptosis. An exciting adjunct to this work is that some dietary constituents also have the ability to act as HDAC inhibitors. Metabolism of food and its individual constituents generates compounds like butyrate, sulforaphane, organosulfur and organoselenium compounds, and indole-3-carbinol derivatives that inhibit HDAC activity and block cancer cell growth. These themes are being developed in a newly funded $8.45 million program project grant from the National Cancer Institute (NCI), entitled "Comparative Mechanisms of Cancer Chemoprevention", which has the following central hypothesis:

"Sulforaphane, indole-3-carbinol, and the cruciferous vegetables from which they derive are effective dietary chemopreventive agents because they alter the pattern of histone modifications (acetylation, methylation, phosphorylation) and HDAC activity in cancer cells, as well as DNA promoter methylation status, thereby de-repressing epigenetically silenced genes that regulate the cell cycle and apoptosis."



LPI investigators Drs. Emily Ho, David Williams, and I will lead three integrated projects that focus on dietary chemoprotection strategies for cancers of the prostate, lung, and colon, as well as lymphomas, and include transgenerational studies in mice. All three projects use facilities and scientific expertise in the centralized Epigenetic/Translational Biomarkers (ETB) Core, headed by Dr. Christiane Löhr. Reporting duties to the NCI will involve an Administrative Core overseen by me, as Program Director. An important and exciting aspect of this work is that it will proceed to clinical trials with human volunteers, testing several of the epigenetic hypotheses in the context of real-world food intake patterns. LPI investigators have been at the leading-edge of research in this area for several years. With this new grant, we hope to go much further along the road to understanding the complex interactions between diet, epigenetics, and cancer prevention. LPI

Developments

Get your name on LPI's new home

The new home for the Linus Pauling Institute is quickly taking shape! After several months of careful work on the basement level, the upper floors of the Linus Pauling Science Center are starting to appear. When finished in the summer of 2011, this state-of-the-art, 105,000-square-foot facility will bring the Linus Pauling Institute to a whole new level of excellence and dramatically enhance the legacy of Linus Pauling. To see our progress, please visit the live webcam at http://webcam.oregonstate.edu/lpsc.

There's still time for you to be a part of this turning point in the history of the Linus Pauling Institute—and to forever make your mark on its new home. By making a gift of $10,000 or more to support the institute's world-class research, your name will be added to the list of donors honored on a wall inside the building. Naming opportunities for offices, classrooms, and labs start at $25,000 and go up to $1 million, and pledges may be paid over five years.

Bequests are another great way you can join the legacy of Linus Pauling. When you designate a gift of $25,000 or more for the institute in your will, your generosity may be honored with a named space inside the building just like a gift that is made in full now. Please consider including LPI in your estate plans—suggested bequest language can be found at http://lpi.oregonstate.edu/giving.html.

For more information about naming opportunities in the Linus Pauling Science Center, contact Kevin Heaney at 541-737-5495 or Kevin.Heaney@oregonstate.edu. And, please remember, we appreciate gifts of any size to support LPI's work.

We are currently reviewing candidates who have applied for the LPI Director of Development position and will have news in the next newsletter. LPI

LPI is grateful for the bequests we have received from the following friends this past year:

Grace Telenius Davis
Louise Marie Sorensen
Joan Dritz

STATE OF OREGON

PROCLAMATION

OFFICE OF THE GOVERNOR

WHEREAS: Linus Carl Pauling, a native son of Oregon, is the only person to have been awarded two unshared Nobel Laureates, for Chemistry and for Peace; and

WHEREAS: Dr. Pauling's efforts to bring about world peace and the banning of nuclear weapons testing, carried out in spite of the disapproval of his own government, established a new base for world peace negotiations; and

WHEREAS: Dr. Pauling's genius in chemistry provided the basis for modern chemistry and set the stage for major discoveries that benefited humankind; and

WHEREAS: Dr. Pauling was unafraid of controversy in his pursuit of the truth and devoted his entire life to the good of humanity; and

WHEREAS: February 28, 2010 is the birth day of Linus Carl Pauling and a time to recognize the life and work of this great American.

NOW, THEREFORE: I, Theodore R. Kulongoski, Governor of the State of Oregon, hereby proclaim **February 28, 2010** to be

LINUS CARL PAULING DAY

in Oregon and encourage all Oregonians to join in this observance.

IN WITNESS WHEREOF, I hereunto set my hand and cause the Great Seal of the State of Oregon to be affixed. Done at the Capitol in the City of Salem in the State of Oregon on this day, February 5, 2010.

Theodore R. Kulongoski, Governor

Kate Brown, Secretary of State

Thank you!

The Linus Pauling Institute gratefully acknowledges grants from the following companies and organizations:

- USANA Health Sciences, Inc. (Salt Lake City, UT), to support human research on health benefits of micronutrients and dietary supplements
- Bayer Consumer Care AG (Basel, Switzerland), to support the Micronutrient Information Center
- Spirit Mountain Community Fund (Grand Ronde, OR) and The Juan Young Trust (Portland, OR), to support the LPI Fitness and Nutrition Study, a local outreach program to improve dietary habits and physical activity in elementary school children. This program is also supported by a generous gift from an anonymous donor.

Linus Pauling Institute

Stephen Lawson, Research Newsletter Editor
Oregon State University
571 Weniger Hall
Corvallis, Oregon 97331-6512

phone: 541-737-5075
fax: 541-737-5077
email: lpi@oregonstate.edu
Internet Web site: http://lpi.oregonstate.edu

Non-Profit Org.
U.S. Postage
PAID
Portland, OR
Permit No. 1006

OSU Oregon State University

GIVING to the Linus Pauling Institute

Gifts in support of research efforts can be made at any time. Checks should be payable to *OSU Foundation for Linus Pauling Institute.* Information on giving is available through the OSU Foundation, **1-800-354-7281,** or by writing to the Institute.

Micronutrient Research for Optimum Health

The Linus Pauling Science Center under construction

To see the progress on our building, please visit our real-time Web cam at http://webcam.oregonstate.edu/lpsc.

LPSC is scheduled for completion in summer 2011.

Look for these informative articles inside!

Special thanks to *Barbara McVicar for editorial assistance and photographs, authors of signed articles, and Dick Willoughby for the logo photograph of Linus Pauling.*