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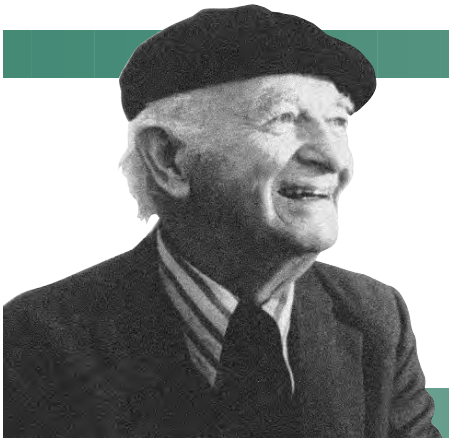

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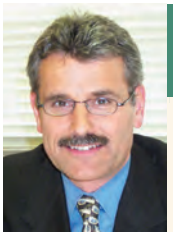
RESEARCH NEWSLETTER



From the Director

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

You may have heard about the recent study examining the effect of antioxidant supplements on death rates published in the February 28, 2007, issue of the *Journal of the American Medical Association*. The study's authors, who are from Denmark, Serbia, and Italy, concluded that antioxidant supplements, in particular beta-carotene, vitamin A, and vitamin E, "may increase all-cause mortality," while "vitamin C and selenium had no significant effect" and their "potential roles on mortality need further study." The study was a meta-analysis, which is a statistical analysis of previously published data pooled together into a single study.

In this study, the authors initially looked at 815 human clinical trials of antioxidant supplements, but only 68 were included in the meta-analysis. A typical clinical trial is a study in which a large group of healthy subjects or patients with a specific disease is randomly divided into two groups that are given either placebo or active treatment (in this case antioxidants) for a long period of time, with neither the participants nor the doctors knowing who is getting which treatment (*i.e.*, the study is double-blinded). The 68 trials deemed acceptable for inclusion in the meta-analysis were divided into either "low bias" or "high bias" trials. While the 47 "low bias" trials showed a 5% increased mortality with antioxidant supplements, the 21 "high bias" trials actually showed a 9% *decreased* mortality with antioxidant supplements. Furthermore, the increased mortality for beta-carotene, vitamin A, and vitamin E supplements was only seen in "low bias" trials, but *not* in all 68 trials combined (except for beta-carotene when given singly). In other words, vitamin E supplementation, for example, was only associated with increased mortality after exclusion of data from "high bias" trials.

"High bias" was defined as "one or more unclear or inadequate quality components" of the trial, *e.g.*, "allocation sequence, concealment, blinding, placebo, and follow-up." This is a subjective cut made by two authors of the paper,

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Diabetes

An Interview with Tammy Bray, Ph.D.
Dean, College of Health and Human Sciences, Oregon State University
LPI Principal Investigator

Q. ***What causes diabetes?***

A. There are two kinds of diabetes—Type 1 and Type 2. Juvenile diabetes, or Type 1, usually begins at an early age and is associated with an acute infection. Type 2 diabetes, or adult-onset diabetes, begins later in life and is associated with diet and lifestyle.

Q. ***How prevalent is diabetes?***

A. Type 1 accounts for between five and ten percent of the diabetes cases, which in total number over 20 million in the U.S. Type 1 is almost like a viral disease, and its incidence is pretty similar in all countries. Type 2 has had a dramatic increase of 80-85% in recent years. Type 2 is preventable. Its prevalence in Western societies increases proportionally with body weight and the BMI (body mass index).

Q. ***Is ethnicity or gender related to diabetes risk?***

A. Those characteristics don't affect risk for Type 1 diabetes, but Hispanics and Blacks are more likely to develop Type 2 diabetes, and the risk for men is greater than for women. If you're prone to obesity, you're more prone to Type 2 diabetes. From an evolutionary perspective, those with thrifty or frugal genes who now eat too much or don't get enough exercise are at risk for Type 2 diabetes. There is a genetic predisposition for Type 2 diabetes, but not for Type 1. When humans were mainly hunters and gatherers, the genetic program favored storing energy because food might not be constantly available. Now, in Western societies we have an abundance of food, and overconsumption leads to energy storage as fat.

Q. ***Is oxidative stress implicated in the development of diabetes and, if so, why?***

A. For Type 1 diabetes, definitely yes. The cause of the disease is multi-factorial and immunity plays a role. Curiously, Type 1 diabetes is often precipitated by an infection like influenza. There's something that triggers the infection and

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with "discussion or arbitration by a third author," as needed. Because of this rather subjective cut, two large trials were excluded from overall consideration that found substantial benefit of antioxidant supplements: a trial in Linxian, China, which observed 6% lower mortality associated with supplemental intake of beta-carotene, selenium, and vitamins A, C, and E and was published in the *Journal of the National Cancer Institute*, and a large trial in Italy called GISSI Prevenzione trial, which found 8% lower mortality with vitamin E supplements and was published in *Lancet*. The authors of the meta-analysis did not explain why these two large, well-designed trials were excluded. They were good enough to be published in the official journal of the National Cancer Institute and the premier British medical journal.

Hence, the arbitrary separation into "high bias" and "low bias" studies and the exclusion of the "high bias" studies from the overall evaluation seem to reveal a bias by the authors in the analysis and interpretation of the data towards showing harm and confirming their own, previously published data. In other words, we have to trust the judgment of two or three authors on whether these two large studies (Linxian and GISSI Prevenzione) are worthy of inclusion in the overall meta-analysis. If these studies were included, none of the effects on increased mortality reported would be significant, except for beta-carotene.

The data on beta-carotene, however, are driven by two large clinical studies, the so-called Alpha-Tocopherol Beta-Carotene (ATBC) Cancer Prevention Study and the Beta-Carotene And Retinol Efficacy Trial (CARET), which showed increased lung cancer incidence in beta-carotene or vitamin A-supplemented heavy smokers, ex-smokers, and workers occupationally exposed to asbestos. This is nothing new, and these two trials have been extensively reviewed, re-reviewed, and covered by the media. Yes, if you smoke, don't take beta-carotene supplements, but much better, quit smoking!

Also, even among the "low bias" trials, many showed decreased mortality with antioxidant supplements. For example, the Supplementation en Vitamines et en Mineraux Antioxydants (SUVIMAX) Vascular Study in France found that supplementation of over 13,000 middle-aged patients with vitamins C and E and selenium resulted in a 22 percent *reduced* mortality after seven years.

Furthermore, clinical trials of dietary antioxidants have serious limitations. They may be the "gold standard" for testing safety and efficacy of pharmaceutical drugs, but are hardly suitable to test dietary compounds, which are metabolized in completely different ways from drugs. In addition, dietary compounds are present in the human body before supplementation occurs, which significantly diminishes the power of the trial to detect a statistically significant effect of the supplement.

Here are some additional limitations and problems with most of the 68 trials reviewed in this meta-analysis:

- In the 47 secondary prevention trials done in patients—as opposed to the 21 primary prevention trials in healthy subjects—the patients were kept on their usual medications (for ethical reasons), which can interfere with or mask the effects of the antioxidant supplements. In addition, most trials tested multiple antioxidants and additional interventions, including a long list of other dietary supplements and pharmaceutical drugs, which again may interfere with or mask the antioxidants' effects.
- Very few, if any, of the trials assessed the levels of oxidative stress in the test subjects. Thus, it was not known at the outset of the study who was under increased oxidative stress and thus might benefit from antioxidant supplementation, nor was it known at the end of the study whether the antioxidant treatment had the intended effect of lowering oxidative stress. It's like doing a cholesterol-lowering trial with a statin drug without ever measuring serum cholesterol in the test subjects. It's impossible to draw firm conclusions from such a poorly designed study!
- The trials pooled together in the meta-analysis used vastly different doses of the various antioxidant supplements, which were given for vastly different time periods. For example, vitamin E supplements ranged from 10 International Units (IU) (22 is the recommended dietary allowance, or RDA) to 5,000 IU (the tolerable upper intake level, or UL, is 1,500). The vitamin A supplements ranged from 1,333 IU (2,333 and 3,000 are the RDA for women and men, respectively) to a whopping 200,000 IU (the UL is 10,000)—high doses of vitamin A (hypervitaminosis A) are well known to have multiple adverse health effects! The duration of the studies ranged from 28 days to 12 years, and the follow-up periods from 28 days to 14 years.
- The causes of death in the studies were vastly different, and often unknown, and any death counted, whether from heart disease, cancer, kidney failure, hip fractures, accidents, or even suicide.

Additionally, mortality data from one 2001 study were misrepresented. The authors of the meta-analysis claimed that there were 13 deaths in the control groups and 17 deaths in the antioxidant groups in that study. Actually, there was only one death in each group.

As Bernadine Healy, former director of the National Institutes of Health and president and CEO of the American Red Cross and current member of the President's Council of Advisors on Science and Technology, put it: "Blenderizing these diverse trials into one giant 232,606-patient-strong study to come up with a seductively simple proclamation is just silly … Sure, statistics can prove anything. But this study violates a cardinal rule of meta-analysis. Pooled studies must be compatible. That means combining apples and apples—or at the least, similar patients and comparable doses and duration of treatments. The first question to ask in evaluating any such study is whether the combination makes sense, both common sense and medical sense, in the first place. On both, the study flunks."

Overall, this is a flawed meta-analysis of flawed data that does not reveal the true health effects of antioxidants, whether beneficial or otherwise, but instead reveals the authors' bias towards showing harm. The analysis is limited to clinical trials of antioxidants, and these trials are well known to have serious limitations. Instead, the totality of evidence from laboratory, animal, and human studies to date shows that antioxidants provide many health benefits, including reduced risk for heart disease and stroke, some cancers, eye diseases, and neurodegenerative diseases, as well as enhanced immunity and resistance to infection. Keep taking your antioxidant supplements, in addition to getting many other micronutrients and phytochemicals from a plentiful intake of fruits and vegetables! LPI

Continued from cover — An Interview with Tammy Bray

inflammation. The pancreas, the organ that produces insulin, may become inflamed and insulin-producing cells, called beta cells, then die, partly as a result of oxidative stress.

Q. ***Why is the pancreas, in particular, affected by the infection?***

A. Some organs are more sensitive to inflammation and oxidative stress. The liver and the lung probably have stronger defense systems. The pancreas, and beta cells in particular, have comparatively less protection. When you get an infection like the flu, free radicals are generated that attack the pathogens and vulnerable cells. Unfortunately, beta cells in the pancreas are very vulnerable to free radical damage.

Q. ***Why are juveniles at risk for developing diabetes as a result of an infection, whereas adults are not?***

A. In young people, the defense system is immature, as measured by substances that protect against oxidative damage, such as superoxide dismutase, glutathione peroxidase, glutathione, and catalase. Older people have higher levels of these protective molecules, so they are less vulnerable.

Q. ***Is there any evidence that good antioxidant status—low oxidative stress—protects against diabetes in children? Has this been studied in animal models?***

A. We haven't done any epidemiological studies, but we did an intervention study with animals. While not all viral infections induce diabetes, some chemicals similar to glucose, such as alloxan, can be used to induce diabetes. Alloxan is very specifically picked up by beta cells in the pancreas and then kills them, stopping the production of insulin. So you prepare the animals when they're young by giving them lots of antioxidants—vitamins E and C, glutathione, or N-acetylcysteine, a precursor of glutathione. Animals that have been fed these antioxidants and then challenged with alloxan are more resistant to Type 1 diabetes. This may be relevant to humans—children with good antioxidant status may be less vulnerable to Type 1 diabetes, but we don't have studies yet to support that.

Q. ***Why does exercise lower the risk of diabetes?***

A. We know that exercise, for whatever reason, makes the receptor for insulin much more sensitive. If you measure insulin in someone who exercises a lot, the concentration is low compared to a sedentary or less active person. When this was observed in the late 1970s, investigators thought something was wrong. They found out that we don't need a lot of insulin. Insulin produced by the pancreas allows muscle to take up the blood glucose after a meal. In very athletic people, the insulin sensitivity is very efficient, so they don't need a lot. In an obese person, more insulin is produced, and it takes more to get the muscle to take up glucose. So the insulin sensitivity is blunted and less efficient. When you have Type 2 diabetes for a long time, there's a drop in insulin production, probably because the pancreas has deteriorated. For whatever reason, physical activity prepares you for high sensitivity of insulin and its cellular receptor. That's why it's important to keep active. When someone first gets diagnosed with Type 2 diabetes, my advice is to avoid sugar and to walk. Even starting with a small amount of walking is very beneficial.

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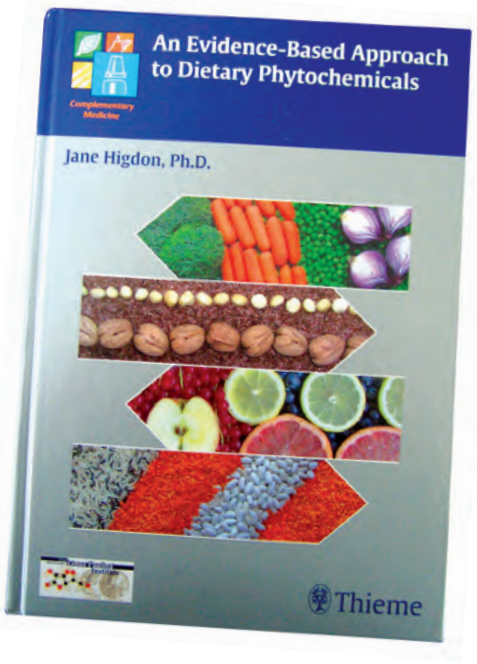
An Evidence-Based Approach to Dietary Phytochemicals

Thieme Medical Publishers published the first of Dr. Jane Higdon's two books, *An Evidence-Based Approach to Vitamins and Minerals*, in 2003, that collects the information on 13 vitamins and 14 nutritionally relevant minerals posted online on the LPI Micronutrient Information Center (http://lpi.oregonstate.edu/infocenter).

In November 2006, Thieme published the companion volume, *An Evidence-Based Approach to Dietary Phytochemicals*, featuring 20 chapters on fruits and vegetables, legumes, cruciferous vegetables, garlic, whole grains, nuts, coffee, tea, essential fatty acids (omega-3 and omega-6), and phytochemicals like carotenoids, chlorophyll, flavonoids, indole-3-carbinol, and resveratrol. Human studies are emphasized, and chapters provide information on metabolism, biological activity, sources, safety, disease prevention, and, when applicable, disease treatment. Each chapter concludes with a summary and extensive references.

As in the previous book, the sections were peer-reviewed by experts, who comprised the Editorial Advisory Board. The 238-page book also contains several appendices, including glycemic index and glycemic load, drug interactions, nutrient interactions, disease reference, and glossary, as well as a comprehensive index. *An Evidence-Based Approach to Dietary Phytochemicals* is dedicated to the memory of its author, Jane Higdon, who died in an accident in May 2006. The book is available from LPI, the publisher, and booksellers for $59.95.



Continued from page 3 — An Interview with Tammy Bray

Q. ***How much exercise do you recommend for people who have just been diagnosed with Type 2 diabetes?***

A. About 30 minutes of exercise a day is for health maintenance. That increases your insulin sensitivity. You can start with ten minutes a day and increase to 20 minutes a day, then to 30 minutes. That may not change your body weight, but you will have a lot of health index changes, like sensitivity to insulin and improved blood lipid profile. Sixty minutes of exercise three times a week is probably best, but if you want to lose weight you have to get 90 minutes and reduce your caloric intake. Exercise also induces antioxidant defense systems. Superoxide dismutase and glutathione peroxidase increase. Exercise may not dramatically reduce your body mass index, but it will increase your health span and improve your quality of life.

Q. ***What is the relationship between insulin and blood sugar and why is chronically elevated blood sugar harmful?***

A. Your body needs energy. The energy that's needed comes from glucose. After a meal, your digestive system converts all the carbohydrates into glucose. The glucose from the carbohydrates you eat has to be absorbed by the muscle and produce energy. Something has to allow the glucose to get into the muscle. Insulin fits into the insulin receptor on cells and permits the glucose in the blood to get into the muscle. Muscle will take it up and store whatever it needs later as glycogen, or burn the fuel in mitochondria to produce chemical energy. In diabetes, the insulin receptor mechanism doesn't work very well, so glucose increases in the blood. Some glucose gets filtered out in the kidney and shows up in urine. This was first observed in dogs without a pancreas. Flies swarmed to their urine because it contained so much glucose.

Q. ***So now the glucose is going into the urine instead of the muscle.***

A. Yes, and that causes a lack of energy. Insulin and glycogen maintain the blood glucose level. When the glucose level becomes too high, it causes the vasculature to become rigid, which increases the risk of heart disease. This process is based on the Maillard reaction, in which advanced glycation end products (AGE) are generated. AGE are toxic and contribute to the risk of heart attacks and other diseases.

Q. ***What happens when the vasculature becomes rigid?***

A. Circulation, especially in the capillaries, becomes impaired. That's why wounds are dangerous in diabetes—the capillaries don't eliminate toxins, raising the risk for tissue damage and amputation of the extremities. The eye is also very sensitive to impaired blood flow. Vascular rigidity is also linked to high blood pressure.

Q. ***What dietary interventions are helpful in managing diabetes?***

A. It helps to eat complex carbohydrates, whole grain products, and fiber in fruits and vegetables. Don't eat simple carbohydrates like sugar or highly processed foods. It takes time to gradually digest and absorb complex carbohydrates or whole grain foods, so your body's reaction to insulin is appropriate. It's not a surge. Simple and processed sugars are readily absorbed in the blood, causing a quicker insulin reaction. If your insulin response doesn't work well, sugar cannot get into the muscle, so you have a high concentration of glucose in the blood.

Q. ***Is that the basis for the glycemic index of foods?***

A. Yes. Glycemic index compares the rise in blood sugar after eating a food to the rise after consuming glucose or white bread.

Q. ***Can Type 2 diabetes be cured by following dietary recommendations?***

A. It can't really be cured, only managed. Over time, the sensitivity of your insulin receptors may increase, but it's critical to manage the disease so that you don't have high blood glucose and the vascular problems that affect the eyes, the feet, and the fingertips.

Q. ***Are antioxidant supplements like vitamin C, vitamin E, and lipoic acid helpful in managing diabetes?***

A. Oxidative stress is associated with diabetes. Lipoic acid has been used to treat diabetic neuropathy, which results from impaired capillary blood flow to the neurons. Lipoic acid also plays a role in energy and protein metabolism. I think that diet and exercise are most important.

Q. ***Is zinc useful in treating diabetes?***

A. I tested zinc. Zinc is an antioxidant, but it also dampens the activity of the immune system, and too much may be toxic. If your baby has a diaper rash you put on zinc oxide to help. But zinc oxide doesn't cure diaper rash; it prevents the damage of the infection.

Q. ***What about chromium?***

A. It's important to maintain a good chromium status and prevent deficiency, but I'm not sure that supplementation helps much in diabetes.

Q. ***Are there any specific foods or beverages that seem to be linked with an increased risk for Type 2 diabetes?***

A. Don't drink soda pop with sugar. It's better to eat fruit than drink fruit juice. Tomato juice is a better choice—low calorie, more fiber, less sugar, and low glycemic index.

Q. ***Are sucrose substitutes acceptable for diabetics?***

A. Fructose is not better because it is converted to glucose in the body. One artificial sweetener is made of hydroxylated phenylalanine. People with PKU can't use that. If you have to use a sweetener, artificial sugar substitutes are okay for most people.

Q. *Why have you studied wound healing?*

A. It seems that so many things are involved—nutrient deficiencies, oxidative stress, and immune function. In diabetics, wound healing is impaired and, as I mentioned, may lead to amputation. The immune system is affected by stress, and that can also cause delayed wound healing. The first stage of wound healing is the blood clot. Of course, if clotting is impaired, you continue to bleed. Too much clotting is also dangerous—it may cause a heart attack or stroke if the clot migrates to other organs. The immune system responds when we have a wound by producing inflammation, which does two things. One, it kills the bacteria at the wound site. It also sends out a signal for cells to grow—to proliferate. The inflammation stage is important, but after these effects have occurred, it needs to subside. Zinc is important for that, as are many other substances. When the inflammation subsides, the next signal triggers proliferation. This produces cells that repair the wound. Then you want the proliferation stopped. Once the new cells add to the skin or some other tissue that's been damaged, the process should stop. If it doesn't, you may have uncontrolled proliferation or cancer.

Q. *What happens in diabetes that impairs this process?*

A. Chronic inflammation is to blame. Inflammation is also linked to prostate cancer. I studied hormonal-induced inflammation, which is involved in prostate cancer. The whole process is just like wound healing except it's much longer. You have a cut on your skin and in a few days new epithelial cells have replaced the wound. If you don't allow the wound to heal, it can become cancerous because the cells keep proliferating. In the prostate, abnormally high hormone levels contribute to the risk of cancer because they are inflammatory.

Q. *How do you study this?*

A. In animals, we can increase testosterone, which increases the incidence of prostate cancer by about 70%. If you also increase estrogen, the incidence increases to 100%. Our hypothesis is that high levels of hormones activate inflammatory molecules like NFκB. The prolonged activation of these molecules causes cell proliferation.

Q. *How is zinc status related to prostate cancer risk?*

A. Zinc is normally found in high concentrations in the prostate and in seminal fluid. We don't know why, but we are working to find out. Zinc deficiency may be a risk factor for prostate cancer.

Q. *Epidemiological studies have found associations between the consumption of tomato products and decreased incidence of prostate cancer. Which phytochemicals in tomatoes might be responsible for this protective effect?*

A. The most popular theory right now is that a carotenoid called lycopene is protective. Other carotenoids may be protective, too. In rats, lycopene is effective in reducing hormone- or carcinogen-induced prostate cancer, but it's not as effective as the whole tomato or freeze-dried tomato powder. Lycopene accumulates in the prostate. It is fat soluble, so the recommendation has generally been to consume tomato products with a little oil to improve absorption.

Q. *Is there any difference in lycopene bioavailability between cooked or raw tomato products?*

A. Lycopene in processed tomato products is more bioavailable, but consumption of any tomato product is probably beneficial.

Q. *What nutritional or lifestyle modifications do you recommend to decrease the risk for prostate cancer?*

A. Levels of prostate-specific antigen, or PSA, are used to monitor prostate cancer risk. If you put men with very high PSA levels on a low-fat anticancer diet that includes tomato products, vegetables, and fruit, like the Mediterranean diet, and also increase their exercise level, they lose weight and their PSA levels drop. If they stop the diet and exercise, their PSA levels go up.

Q. *In addition to conducting research, you are Dean of the College of Health and Human Sciences. What activities do you encourage in the College?*

A. Well, collaboration is my philosophy and guiding principle. I think people shouldn't do anything in isolation. Collaboration is not simply doing things together; it means solving problems in multiple ways. Different scientists have different perspectives. When we collaborate, there is synergy and the whole becomes much more than the sum of the parts. So I am motivated to bring faculty together. I want to help the junior faculty in my college to be successful, help them to write grants and collaborate. LPI

Two Faces of Inflammation

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

What is inflammation?

Inflammation is the immune response of tissues due to bodily injury. Clinical characteristics of acute inflammation include pain, heat, swelling, and redness at the site of the injury. Inflammation may also involve loss of function of the involved tissues. This type of acute inflammation is normally a localized, protective response following trauma or infection. However, if the agent causing the inflammation persists for a prolonged period of time, the inflammation becomes chronic. Chronic inflammation can result from a viral or microbial infection, environmental antigen (*e.g.*, pollen), autoimmune reaction, or persistent activation of inflammatory molecules.

The inflammatory process involves a complex biological cascade of molecular and cellular signals that alter physiological responses, ultimately resulting in the familiar clinical symptoms. At the site of the injury, cells release molecular signals that cause a number of changes in the affected area: dilation of blood vessels, increased blood flow, increased vascular permeability, exudation of fluids containing proteins like immunoglobulins (antibodies), and invasion by leukocytes (white blood cells). Several different types of leukocytes, including granulocytes, monocytes, and lymphocytes, are involved in the inflammatory cascade. The cellular mediators of acute and chronic inflammation differ somewhat and are discussed below.

How does acute inflammation differ from chronic inflammation?

Acute Inflammation

Acute inflammation is a normal process that protects and heals the body following physical injury or infection. Acute inflammation involves local dilation of blood vessels as well as increased vessel permeability to improve blood flow to the injured area. At the site of an infection or injury, mast cells, platelets, nerve endings, endothelial cells, and other resident cells release signaling molecules and chemoattractants that recruit leukocytes to the affected area. Neutrophils, a type of granulocyte, are the first leukocytes to appear at the injured site. These cells phagocytose (engulf) and kill invading microorganisms through the release of non-specific toxins, such as superoxide radicals, hypochlorite, and hydroxyl radicals; these reactive oxygen species (ROS) kill pathogens as well as adjacent cells, sick and healthy alike. Neutrophils also release cytokines, including interleukin (IL)-1, IL-6, tumor necrosis factor (TNF)-α, gamma interferon (INF-γ), and others. Such pro-inflammatory cytokines in turn induce the liver to synthesize various acute phase reactant proteins and also induce systemic inflammatory responses (*e.g.*, fever and leukocytosis—a rise in the number of white blood cells). Neutrophils are short-lived and are thus primarily involved in the early stages of inflammation.

Chronic Inflammation

If the stimulus persists, inflammation can last days, months, and even years. Chronic inflammation is primarily mediated by monocytes and long-lived macrophages; monocytes mature into macrophages once they leave the bloodstream and enter tissues. Macrophages engulf and digest microorganisms, foreign invaders, and senescent cells. Macrophages release several different chemical mediators, including IL-1, TNF-α, and prostaglandins, that perpetuate the pro-inflammatory response. At later stages, other cells, including lymphocytes, invade the affected tissues: T lymphocytes kill virus-infected cells and B lymphocytes produce antibodies that specifically target the invading microorganisms for destruction.

Macrophages and other leukocytes release ROS and proteases that destroy the source of inflammation; however, damage to the body's own tissues often results. In fact, tissue damage is a hallmark of chronic inflammation. Another characteristic of chronic inflammation is repair of the damaged tissue by replacement with cells of the same type or with fibrous connective tissue. An important part of the inflammatory process involves local angiogenesis—the development of new blood vessels. In some instances, the body is unable to repair tissue damage, and the inflammatory cascade continues. Chronic inflammation is abnormal and does not benefit the body; in fact, chronic inflammation is involved in a number of disease states (see below).

Are specific tissues or organs especially vulnerable to the effects of inflammation?

Tissue damage that occurs during the inflammatory response must be actively repaired. Repair capabilities of the tissues within the body vary greatly because the cells have different regenerative abilities. For instance, cells with little to no regenerative capacity include neurons, cardiac cells, and skeletal muscle cells. Tissues comprised of these cells would be especially vulnerable to effects of inflammation. In contrast, skin cells are labile because they continue to proliferate throughout life; thus, wounds to the skin are often easily healed.

How is inflammation detected?

The inflammatory response involves countless mediators, some of which are used as clinical markers of inflammation or inflammatory diseases. The table on the next page lists a few commonly used biomarkers of systemic inflammation. C-reactive protein (CRP), an acute-phase reactant protein synthesized in the liver, is the prototypic clinical biomarker of cardiac-related inflammation and is also a general marker of inflammation. Clinically, the most common tests to diagnose inflammation include measuring erythrocyte sedimentation rate (ESR), white blood cell count, and albumin levels. High ESR, high white cell counts, and low albumin are markers of inflammation. All of these tests are nonspecific; that is, an abnormal result might result from a condition unrelated to inflammation. Various cytokines and adhesion molecules are not often used in clinical settings primarily because such tests would not identify the source of inflammation within the body. However, such biomarkers are frequently used in basic scientific studies to investigate the cellular and molecular processes involved in the pathogenesis of inflammation-related diseases.

Biomarkers of inflammation

Acute-phase reactant proteins
(CRP, SAA, vWF antigen, fibrinogen)

White cell count, ESR, albumin

Cytokines (IL-1β, IL-6, IL-18, TNF-α)

Adhesion molecules (E-selectin, P-selectin, ICAM-1, VCAM-1)

Abbreviations: CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; ICAM-1, intercellular adhesion molecule-1; IL, interleukin; SAA, serum amyloid A protein; VCAM-1, vascular cell adhesion molecule-1; vWF, von Willebrand factor

What diseases are associated with inflammation?

Several human diseases are inflammatory in nature, including asthma, Crohn's disease, rheumatoid arthritis, polymyalgia rheumatica, tendonitis, bursitis, laryngitis, gingivitis, gastritis, otitis, celiac disease (gluten intolerance), diverticulitis (infection of the diverticula in the colon), and inflammatory bowel disease. Additionally, a number of chronic diseases have inflammatory components, such as atherosclerosis, obesity, diabetes, cancer, and perhaps even Alzheimer's disease. The causes of several of these diseases are unknown, and the role of inflammation in disease pathogenesis is under investigation. For instance, it is known that adipose tissue secretes several inflammatory factors (adipocytokines) and that obesity is associated with macrophage infiltration in adipose tissue; however, the exact role of inflammation in the pathogenesis of obesity is currently unknown.

What dietary and lifestyle interventions may be useful to decrease inflammation?

Dietary components may modulate inflammatory responses within the body (see the table at right). Overall, studies suggest that diets rich in saturated fats, *trans* fats (hydrogenated or partially hydrogenated oils), and high glycemic index foods stimulate inflammation. In contrast, adherence to a Mediterranean-style diet has been shown to reduce inflammation. A Mediterranean-style diet is rich in monounsaturated fatty acids from olive oil, fruits and vegetables, nuts, beans, and whole grains. In addition, a Mediterranean-style diet emphasizes consuming alcohol in moderation. Moderate alcohol consumption has been associated with a reduction in inflammation.

Essential fatty acids play a role in the body's inflammatory processes. Increasing dietary intake of the omega-3 fatty acids found in oily fish and fish oils—eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)—generally decreases several markers of inflammation, whereas increasing dietary intake of omega-6 fatty acids increases inflammatory markers. The ratio of omega-6 to omega-3 fatty acids in the typical Western diet is about 16:1, yet it is estimated that humans evolved on a diet with an omega-6 to omega-3 fatty acid ratio of about 1:1. Decreasing this ratio will likely reduce the prevalence and severity of various inflammatory conditions observed in Western societies.

Particular vitamins, minerals, and phytochemicals may also have therapeutic benefit in inflammatory diseases, but efficacy might depend on the specific disease state. A few reports suggest that the spices turmeric (curcumin) and ginger, as well as the herb *Boswellia serrata*, may inhibit the pro-inflammatory lipooxygenase or cyclooxygenase pathways; however, scientific data supporting anti-inflammatory properties are largely lacking. Catechins, polyphenolic compounds found in tea, have both antioxidant and anti-inflammatory effects. Further, data from animal models suggest that alpha-lipoic acid, when provided as a dietary supplement, has anti-inflammatory properties.

In addition to specific dietary components, achieving or maintaining a healthy body weight is important to prevent or control chronic inflammatory diseases. For instance, elevated CRP levels have been linked to obesity, and weight loss has been shown to decrease CRP levels. Weight loss most consistently results from both reducing daily caloric intake and increasing physical activity. Animal and human studies have found that various forms of exercise decrease both acute and chronic inflammation, as measured by reductions in CRP and particular cytokines. Furthermore, smoking cessation has been reported to decrease CRP, fibrinogen, and white blood cell count—all biomarkers of inflammation.

Dietary and lifestyle factors associated with inflammation

Increase inflammation	Reduce inflammation
Trans fatty acids	Omega-3 fatty acids
Saturated fatty acids	Low cholesterol diets
High glycemic index foods	Low glycemic index foods
Excessive alcohol	Dietary fiber
Smoking	Arginine-rich foods (fish, nuts)
Excessive exercise	Moderate alcohol intake
	Physical activity

Are non-steroidal anti-inflammatory drugs (NSAIDs) considered safe?

NSAIDs like aspirin and ibuprofen are commonly used to relieve pain and control inflammation. Most NSAIDs nonspecifically inhibit one or more isoforms of cyclooxygenase (COX), an enzyme that catalyzes the formation of pro-inflammatory prostaglandins and thromboxanes. It is thought that the nonselective nature of NSAIDs causes adverse upper gastrointestinal effects in some regular NSAID users. Also, because NSAIDs inhibit thromboxanes, users experience decreased blood clotting ability, which could have clinical significance (*e.g.*, increased risk of hemorrhagic stroke). Further, use of COX-2 specific NSAIDs, such as Celebrex and Vioxx, may have low gastrointestinal risks, but studies indicate increased risk of cardiovascular events with use of these drugs. Use of any type of NSAIDs should be closely supervised by a medical provider who examines the potential risks as well as therapeutic benefits. LPI

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

Audrey L. Blanchard
Nancy J. Bradford
Evelyn H. Bullock
Dorothy Epstein
John F. Holterhoff
David B. Holtzman
Arthur Kahn
Marian M. Kiger
Sarah Kupchik
Rosanne McVay
Karla Pepe
Martha A. Winn

The Linus Pauling Institute Survey

A little more than one year ago, LPI completed the analysis of its first demographic survey. The Institute had never before made a systematic survey of its constituency, and we were curious and excited to learn more about our donors and newsletter readers.

To help us conduct a scientific survey, we engaged Dr. Virginia Lesser, Associate Professor of Statistics and Director of the Survey Research Center (SRC) at Oregon State University. For its clients, the SRC designs, administers, and analyzes surveys. For the LPI survey, the SRC selected a random sample of about half the people on LPI's mailing list. About 30% of the mailed survey questionnaires were completed and returned. The data that follows are based on those responses and are reasonably representative of LPI's constituents.

Characteristics of the respondents:

Male	**53%**	Female	**47%**
Over 45 years old	**86%**	Over 65 years old	**51%**
College graduate	**73%**	Advanced degree	**50%**

Responses to questions about behavior:

Internet user	**70%**
Visit LPI Web site	**33%**
Visit Research Newsletter	**71%**
Visit Micronutrient Information Center	**56%**
Found it useful	**99%**
Read latest LPI Research Newsletter	**64%**
Found it understandable	**92%**
Found it informative	**92%**
Found it interesting	**92%**
Familiar with LPI's Rx for Health	**39%**
Take supplemental vitamin C	**89%**
500 mg/day	**25%**
1,000 mg/day	**20%**
more than 1,000 mg/day	**29%**
Take supplemental vitamin E	**84%**
200 IU/day	**16%**
400 IU/day	**42%**
more than 400 IU/day	**15%**
Take supplemental B vitamins	**69%**
Take a multivitamin	**79%**

Main interests of the respondents:

Disease prevention by diet and supplements	**82%**
Health benefits of specific foods/beverages	**79%**
Supplement benefits	**77%**
Disease treatment by diet and supplements	**76%**
Nutrition and aging	**73%**
Supplement safety	**69%**
Nutrition and immune function	**69%**
Nutrition and heart disease/stroke	**68%**
Nutrition and cancer	**68%**
Nutrition and neurodegenerative diseases	**60%**
Nutrition and osteoporosis	**56%**
Exercise benefits	**54%**

Increasingly, people become acquainted with LPI through Internet searching that often links to sections on the Micronutrient Information Center (MIC) or to the biography of Linus Pauling on our Web site. Other analyses indicate that the LPI Web site has over 900,000 "hits" (visits of very short length) and about 130,000 "sessions" (visits of longer duration) every month. Almost three-quarters of these online visits are to the MIC. These figures have increased substantially over the last few years. A search using the Internet search engine Google with the keywords "Linus Pauling" returns over 1,800,000 links to Web pages in which the term "Linus Pauling" appears. The first Web page on that long list is the biography of Linus Pauling posted on the LPI Web site. Similarly, a search for "vitamins" returns over 76,000,000 Web pages, with LPI at the number seven position.

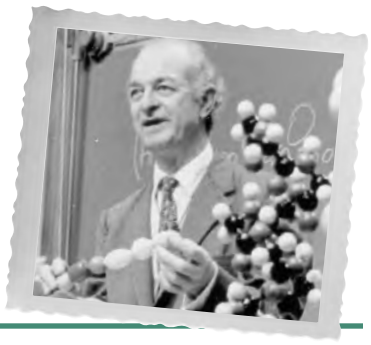
Of the respondents who have been receiving information about LPI for over five years, most learned about LPI through a book or article by or about Linus Pauling. Of the respondents who have received information about LPI for under five years, about one-third learned about LPI through the Internet, demonstrating the increasing power of the Web to inform.

We think that the results of the survey indicate that our public outreach programs are successful. Almost everyone who uses the online Micronutrient Information Center finds it useful, and the Research Newsletter appears to be understandable, informative, and interesting to its readers.

Please contact us with any suggestions for ways to improve the educational services that we offer.

Linus Pauling Day

Ted Kulongoski, Governor of Oregon, proclaimed February 28th, Linus Pauling's birthday, "Linus Carl Pauling Day." Dr. Pauling was recognized in the Proclamation for his "efforts to bring about world peace and the banning of nuclear weapons testing" and for his "genius in chemistry [that] provided the basis for modern chemistry and set the stage for major discoveries that benefited humankind." Governor Kulongoski also noted that, "Dr. Pauling was unafraid of controversy in his pursuit of the truth and devoted his entire life to the good of humanity."



Selenoprotein W in Embryonic Development and Oxidative Stress

Chrissa Kioussi, Ph.D.
Assistant Professor, OSU Department of Pharmaceutical Sciences

Selenium is an essential element for human health. There are places in the world, such as China, where selenium deficiency is still a problem. In lambs and calves, selenium deficiency can cause white muscle disease and, in humans, Keshan cardiomyopathy, deforming arthritis in Kashim-Beck disease, and myxedematous cretinism. Selenium-deficient diets in animals can modify the course of viral infections and can cause mutations within the virus that create new, more virulent strains. Additionally, many studies have demonstrated that selenium supplementation has anticancer effects when given in pharmacological amounts.

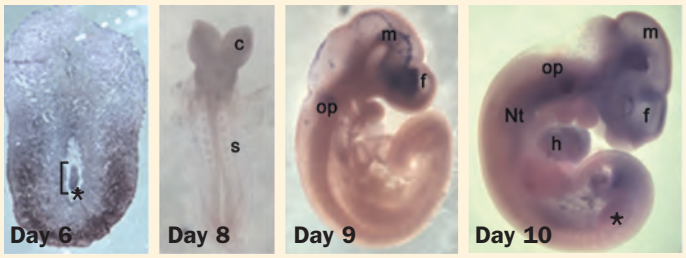
Selenium is involved is several cellular processes, including regulation of thyroid hormone metabolism, regulation of the redox state of cells, cancer prevention, and immune function. Low blood selenium levels are associated with several pathologies, including cancer, cardiovascular disease, and mental illness. It is believed that selenium exerts its protective effects by preventing oxidative damage. Neuronal and neuromuscular disorders, including Alzheimer's, Parkinson's, amyotrophic lateral sclerosis, stroke, and Duchenne Muscular Dystrophy, are diseases associated with free radicals and oxidative stress. The synthesis of the selenocysteine-containing proteins is an essential prerequisite for fetal development and a healthy life.

So far, 25 mammalian selenoproteins have been identified that contain selenium in the form of the amino acid selenocysteine. Availability of selenium is a critical, limiting factor for the synthesis of selenocysteine. Most of the selenoproteins for which an enzymatic function has been determined possess antioxidant activities, suggesting that human diseases associated with selenium deficiency may be attributed to increased oxidative stress and alterations in redox signaling.

Selenoprotein W (SePW) is a mammalian protein of 87 amino acids in length. SePW is highly conserved among mammals—it varies little between species. SePW binds to an important endogenous antioxidant, glutathione, in cells. It was originally purified in the early 1990s in the laboratory of Dr. Phil Whanger at Oregon State University and found to be missing in selenium-deficient lambs suffering from white muscle disease. Expression of SePW in the brain is preserved even in selenium-deficient animals, which suggests a crucial role in nervous system development and function. Recent studies have indicated that SePW is a molecular target of methylmercury, an environmental toxicant. Methylmercury decreases SePW and, consequently, is associated with intracellular glutathione depletion in neuronal cells. When SePW is increased in epithelial ovary and lung cancer cells, these cells exhibit a markedly reduced sensitivity to hydrogen peroxide.

We recently found that SePW is present in newly implanted embryos in mice. SePW was found in the ectodermal cells of the egg cylinder stage, which give rise to the neuroepithelium of the cephalic neural folds, which, in turn, form parts of the brain (cortex, thalamus, and hypothalamus) and spinal cord. SePW was also found in the heart and the somites, the mesodermal structures that give rise to skeletal muscles. We observed that SePW levels gradually increased during the second week of gestation in mice (equivalent to the second trimester in humans) and dramatically increased by the end of the gestation period as a result of the high proliferation rate of brain, heart, and skeletal muscle tissues. This functional profile of SePW suggests its involvement in the transition from the maternal genome to the embryonic genome and in the development of organ systems, such as brain, heart, and skeletal muscles.

Selenoprotein W in the developing mouse embryo



SePW was first detected at the deciduas of the implanted embryo at day 6 and in the ectodermal cells (asterisk) of the implanted embryo (bracket). At day 8 SePW was expressed in cephalic neural folds (c) and the somites (s). At day 9, SePW was detected in the forebrain (f), midbrain (m), neural tube and otic pit (op). At day 10, SePW was detected in the forebrain, midbrain, neural tube (Nt), heart (h), and hind limb (asterisk).

In cell culture studies, we found that SePW levels were increased in the proliferating myoblasts that give rise to muscle tissue, with a subsequent decrease during further differentiation, suggesting a role in development and growth. When proliferating myoblasts were exposed to hydrogen peroxide, levels of SePW decreased after 30 minutes. After two hours, SePW levels decreased by 56%. Inhibition of glutathione synthesis in these cells also dramatically decreased SePW levels. This immediate response of SePW in cells treated with hydrogen peroxide suggests that SePW is involved in the oxidative metabolic pathways and functions as an antioxidant to maintain proper muscle function. Other investigators found that SePW is decreased by cadmium. Glutathione is implicated in the anti-cadmium defense through a transcription factor called MTF-1. MTF-1 binds to sequences in DNA that code for the SePW promoter. Given the effect of methylmercury, hydrogen peroxide, and cadmium on SePW, it appears that SePW can be used to monitor immediate oxidative and toxic stress.

Another selenoprotein, Selenoprotein N (SEPN1), is also found in muscles and linked to congenital muscular dystrophies. SEPN1 is localized in the endoplasmic reticulum in cells, which is involved in calcium sequestration and muscle contraction. Calcium sequestration is lost in the sarcoplasmic reticulum in the muscles of animals with white muscle disease, where SePW is absent. Therefore, it is likely that both forms of selenium are linked to both muscle development and disease. LPI

10TH ANNIVERSARY COLLOQUIUM

On November 1st and 2nd of 2006, the Linus Pauling Institute celebrated its 10th anniversary at Oregon State University. As part of that celebration, the Institute presented a Research Colloquium where the Principal Investigators provided an overview of their current research.

Synopses provided by Sandra Uesugi, OSU Environmental Health Sciences Center

Joseph Beckman, Ph.D.
Ava Helen Pauling Chair and Principal Investigator, Linus Pauling Institute, Professor of Biochemistry and Biophysics, Oregon State University

"Free radicals, antioxidants, and Lou Gehrig: The brain is a terrible thing to waste"

Despite a remarkable recent increase in research spending by the National Institutes of Health (NIH) to investigate human health and disease, health care costs in the U.S. continue to rise by 15% per year and will rise from 15% to 23% of the GNP in the next ten years with the aging of the population. Primary prevention is by far the most cost-effective means to control health care costs and to improve public health. The goal of primary prevention is to improve human health and reduce susceptibility to disease before patients end up in the hospital needing expensive medical treatments.

According to the National Institute of Environmental Health Sciences (NIEHS), environmental stresses, age, and individual susceptibility are major factors influencing human health and disease mechanisms. Our health status is a delicate balance between damage and repair mechanisms. When we are young, our bodies are better able to handle damage from carcinogens (cancer-causing agents), infection, UV radiation, oxidative stress, and other endogenous and environmental insults. However, as we age, our repair systems become less able to manage these stresses. Aging, in simple terms, is the body's decreasing ability to deal with stress. We do not have control over some individual susceptibility factors, such as age, gender, and genetics. Others, such as diet and lifestyle, can be modified. At the Linus Pauling Institute, we are examining the roles of micronutrients and their abilities to improve our individual resistance to disease.

Antioxidants have been linked to protective health benefits in several population-based research studies in the development of neurological and neurodegenerative diseases. A long-term study tracked almost one million American adults for over 15 years. One of the factors studied was regular vitamin E intake. Compared to people who never took vitamin E supplements, individuals taking vitamin E for two to ten years had a 41% decreased risk of developing amyotrophic lateral sclerosis (ALS, also known as Lou Gehrig's disease). For individuals regularly taking vitamin E for over ten years, the risk of developing ALS was reduced by 62%. This suggests that regular, long-term intake of vitamin E significantly reduces the risk of developing ALS. Another study of elderly residents of Cache County, Utah, showed a decreased incidence of Alzheimer's disease associated with regular intake of vitamins E and C taken simultaneously. Delaying the onset of Alzheimer's by just five years is estimated to save the equivalent of the entire NIH budget in health care costs.

Neurological diseases, such as ALS, Alzheimer's, Parkinson's, and mad-cow disease, share many common features. All involve the improper configuration of proteins and perhaps the binding of copper and zinc in inappropriate locations.

An important antioxidant protective enzyme related to ALS is superoxide dismutase or SOD. SOD works to remove superoxide, a free radical compound. While superoxide is not the most dangerous compound itself, it can combine with nitric oxide (a compound important for the relaxation of blood vessels) to form peroxynitrite, a very destructive oxidative compound. We and others have found that a certain number of ALS cases are related to mutations of the SOD gene. These mutations slightly weaken the ability of SOD to hold on to its zinc atom in the enzyme's active site, leading to conditions that foster the formation of superoxide and peroxynitrite.

Our research shows that peroxynitrite affects astrocytes, cells in the brain that support motor neurons (nerves responsible for muscular movement). This leads to the degeneration of motor neurons as observed in patients with ALS. Our research suggests that peroxynitrite does not simply damage astrocytes. The destructive cycle propagates itself by activating the astrocytes, causing these formerly supporting cells to become destructive to even more motor neurons.

Our collaborators in the Luis Barbeito lab in Uruguay have shown that a protein called Nuclear factor-erythroid 2-related factor 2 (Nrf2) and increased glutathione (an antioxidant produced in the body) can reduce the effects of damaging astrocytes on motor neurons. This mechanism also has implications on Parkinson's and Alzheimer's, since those diseases have similar pathologies. LPI

Fred Stevens, Ph.D.
Principal Investigator, Linus Pauling Institute Assistant Professor of Medicinal Chemistry, Oregon State University

"Bioconjugation of lipid peroxidation products: A new role for vitamin C?"

In some of my earlier studies, I studied the colored compounds in henna, a traditional skin and hair dye. We were interested in lawsone, the compound that gives henna its orange color. Henna is traditionally prepared by mixing ground henna powder with water to make a paste, which sits for several hours before use. After analyzing traditionally prepared henna-water paste and a methanol extract of henna that did not turn orange, we noticed that lawsone is present in henna in a pro-dye form (dihydro-lawsone glucoside), which is enzymatically converted to lawsone in the water paste. We also noticed that vitamin C, which naturally occurs in the henna plant, reacts with lawsone and creates a stable, new water-soluble vitamin C conjugate.

We wondered if this reaction with vitamin C also occurs in the human body. The oxidized form of lawsone has chemical reactivity similar to oxidized lipid (fat) compounds produced under conditions of oxidative stress. These oxidized lipids can lead to oxidative damage in the body, especially to DNA and to proteins. Similar to what we observed in the henna plant, we found that vitamin C reacts with oxidized lipids to form vitamin C conjugates in test-tube reactions, using vitamin C concentrations normally present inside cells. We are currently investigating the

biological relevance of this previously unrecognized property of vitamin C by analyzing blood plasma and tissue homogenates. It is well known that oxidized lipids, which play a role in the initiation and progression of atherosclerosis and other age-related diseases, are detoxified by conjugation with glutathione, a natural antioxidant produced by the body. Our research will test the hypothesis that vitamin C contributes to the detoxification and elimination of oxidized lipids through conjugation with vitamin C. LPI

Tory Hagen, Ph.D.
Principal Investigator, Linus Pauling Institute
Associate Professor of Biochemistry and Biophysics, Oregon State University

"Increasing 'healthspan' through age-essential micronutrients"

The American lifespan has increased to an average of 80 years, compared to 50-55 years about 100 years ago. In the U.S., there are now more people over age 85 than people over age 65 just one hundred years ago.

We introduced the concept of "healthspan"—the years of our lives filled with good health and vitality. Despite the fact that Americans are living longer, we are not necessarily living well in our later years. A majority of people over the age of 65 years report having one or more chronic health problems. How do we bridge the gap between lifespan and healthspan?

My research team is investigating "age-essential" micronutrients. These are nutrients that decline in the body with age, negatively affecting normal health functions. These compounds also improve the capacity of the body to cope with a variety of oxidative, environmental, and toxicological stresses.

One micronutrient we are currently investigating is L-carnitine. This compound is vital to the function of mitochondria, the "cellular powerhouses" involved in creating energy for cells. As we age, L-carnitine levels decrease in cells. We have done studies on older rats (age 24 months) and fed them acetyl-L-carnitine (ALCAR), a natural form of L-carnitine that is more bioavailable than L-carnitine itself. These older rats showed significant increases in physical activity, balance, short-term memory, and energy at the cellular level.

However, ALCAR does not improve the body's ability to deal with oxidative stress or improve the body's stress response.

To complement ALCAR, we are also studying alpha-lipoic acid (LA), another micronutrient that helps the body deal with stress. LA can act as an antioxidant and is also used in the mitochondria to convert food nutrients into energy. We have shown that LA administration to older rats results in lower oxidant levels, increased levels of endogenous cellular antioxidants, and improved stress response to chemicals. LA helps turn on the body's built-in stress response systems, which are not as strongly activated as the body ages. Subclinical inflammation and triglycerides were also decreased in older rats fed LA.

Animals fed LA showed only temporary increases of LA in the plasma. A time-release dose may help maintain levels in the body. Cruciferous vegetables (broccoli, kale, collard greens, and Brussels sprouts) are good sources of LA. LPI

George Bailey, Ph.D.
Principal Investigator, Linus Pauling Institute
Distinguished Professor, Oregon State University

"Chlorophylls in the prevention of cancer: From animal models to humans"

The human cancer risk can be substantially lowered by modifying our diet. Many food compounds have been shown to lower cancer risks in animals. However, not many studies have looked at dietary factors and cancer risk in humans.

Our current research is examining the effect of chlorophyll (the green compound found in plants) and chlorophyllin (a water-soluble version of chlorophyll) in reducing cancer risk.

Aflatoxin B1 (AFB1) is a toxin produced by a fungus, which grows on grains and peanuts. AFB1 is known to cause DNA damage in the liver and can lead to liver cancer. AFB1 is a problem in less developed countries and areas where grains are stored in warm, moist areas, which encourages the growth of the fungus that produces AFB1.

In prior animal studies, we found that chlorophyllin reduces the amount of AFB1 that is absorbed in the digestive system, resulting in less AFB1 reaching the liver, less liver DNA damage, and thus less liver cancer. DNA repair systems in our bodies work to remove most of the DNA damage caused by AFB1. The damaged DNA products can be detected in urine.

One in ten adults in the Yangxi delta region of rural China dies of liver cancer. This extraordinary statistic is due to a combination of two risk factors—chronic hepatitis B virus infection and unavoidable dietary intake of AFB1 from moldy corn and other grains. In a large cooperative project, we were able to examine the cancer protective effects of chlorophyllin in humans. We provided a chlorophyllin tablet with each meal to volunteers in the city of Quidong for four months. For those volunteers who received doses of chlorophyllin, we observed a 55% reduction of DNA-damaged products in the urine as compared to those who received a placebo. This suggests that chlorophyllin reduces the amount of AFB1 absorbed from the human digestive system.

To expand upon our findings, we are currently doing a small pilot study on human volunteers who are given very small doses of AFB1, doses equivalent to 5% of what the U.S. FDA allows in one peanut butter sandwich. Using very sensitive analytical equipment, we are able to track AFB1 absorption and elimination from the human body, something that has never been done in people. We are studying the absorption of oral doses of AFB1 alone, AFB1 plus chlorophyll, and AFB1 plus chlorophyllin. Initial results from a single trial showed that chlorophyll reduced the amount of AFB1 absorbed into the blood stream, which suggests that less AFB1 reached the liver. Chlorophyllin also reduced the amount of AFB1 absorption, but to a lesser degree. We have only begun these studies, and the analysis will be completed next year. LPI

continued on page 12

Continued from page 11 — 10th Anniversary Colloquium

David Williams, Ph.D.
Principal Investigator, Linus Pauling Institute
Professor of Environmental and Molecular Toxicology, Oregon State University

"Dietary supplementation of mothers during pregnancy and nursing protects their offspring from cancer in later life"

Cancer is the second highest cause of death in infants and children, second only to accidents. Lymphoma and leukemia are the most common childhood cancers.

Part of our work focuses on dibenzo[a,l]pyrene (DBP), a carcinogen found in tobacco smoke and charbroiled meats. Our animal studies show that DBP causes lymphoma and lung tumors in infants born to mothers who were exposed to DBP. Fetuses and newborns are exposed to DBP through the placenta and mothers' breast milk.

If a mother's behavior can expose her unborn child to carcinogens, then we suspect that a mother can also protect her child through her diet and lower her child's chances of developing cancer. Our research focuses on phytochemicals (compounds found in plants) and their ability to block the activation of carcinogens within the fetus and newborns.

One of our studies examines the ability of indole-3-carbinol (I3C) to protect offspring from cancer caused by a mother's exposure to DBP. I3C is found in cruciferous vegetables (broccoli, cauliflower, cabbage, and Brussels sprouts). In studies with mice, we demonstrated that I3C in the mothers' diets significantly protected their offspring from death due to lymphoma and reduced the number of lung tumors in offspring by one-third.

This is the first research that shows cancer protection of offspring through the mother's diet. It is also striking that the offspring were protected from cancer up to middle age even though they were not given I3C once they were weaned from the mother's breast milk. The doses given to pregnant and nursing mothers in this animal study were equivalent to about twice the amount that a person would consume if taking a commercially available form of I3C dietary supplement.

We also wanted to look at mothers' dietary protection of genetic causes of cancer in their offspring. Our bodies contain two copies of a tumor suppressor gene. When one or both genes are damaged, tumors and cancer are much more likely to develop. Our research showed that I3C greatly decreased death in animals with one damaged tumor suppressor gene, but it did not protect animals with damage to both copies of the gene. Again, only mothers were fed I3C, and offspring were not exposed to I3C except through the placenta and breast milk. This research clearly demonstrates that a maternal diet containing I3C protects offspring from genetic causes of cancer.

We are also looking at the cancer protection of offspring by mothers drinking green tea. Again we are focusing on offspring lymphoma caused by mothers' exposure to dietary DBP. Our research shows that green tea may provide some protection of offspring death due to DBP-induced lymphoma. Caffeine alone (equal to green tea concentrations) provides more protection to offspring, and EGCG (a green tea phytochemical) provides no protection. Offspring from all the treatments were protected from DBP-induced lung cancer in their later lives (middle age), and EGCG was the most protective. LPI

Rod Dashwood, Ph.D.
Principal Investigator, Linus Pauling Institute
Professor of Environmental and Molecular Toxicology, Oregon State University

"Genetic and epigenetic approaches to cancer prevention and therapy by dietary agents"

Our research examines protective compounds found in foods that might prevent or lower the incidence of colorectal cancer.

Cooking meat at high temperatures can produce heterocyclic amine mutagens. The U.S. National Toxicology Program recently classified these compounds as "reasonably likely to be human carcinogens." They cause DNA damage and, in some cells, this leads to overproduction of a protein called beta-catenin, resulting in colon cancer. A compound found in tea has been shown to reduce levels of beta-catenin in human colon cancer cells *in vitro* (experiments performed in cultured cells). This compound is present in all forms of tea (white, green, oolong, and black), but the highest concentrations are found in white and green teas. The compound in question, epigallocatechin-3-gallate (EGCG), typically is not found in herbal teas. Our animal studies show that white tea given in place of the drinking water resulted in fewer pre-cancerous lesions induced by cooked-meat mutagens in the rat colon and lowered spontaneous intestinal polyps in mice.

Another pathway leading to colon cancer development involves the deactivation of tumor suppressor genes. As their name suggests, tumor suppressor genes prevent cells from developing into tumors and potentially leading to cancer. These tumor suppressor genes can be deactivated in cancer cells by an enzyme called histone deacetylase (HDAC). We wanted to know if food compounds can act as HDAC inhibitors, thereby turning on these silenced tumor suppressor genes. One food compound called sulforaphane, which is found in cruciferous vegetables, was an effective HDAC inhibitor blocking the growth of human colon and prostate cancer cells in cell culture. Sulforaphane also decreased HDAC activity in various mouse tissues after feeding in the diet and inhibited HDAC activity in circulating blood cells of humans after they consumed sulforaphane-rich broccoli sprouts. There were fewer intestinal polyps in mice given sulforaphane or EGCG alone, compared to control animals, but sulforaphane and EGCG in combination did not appear to act synergistically in preventing tumor formation in mice. LPI

Emily Ho, Ph.D.
Principal Investigator, Linus Pauling Institute
Assistant Professor of Nutrition and Exercise Sciences, Oregon State University

"Dietary influences on DNA integrity and prostate cancer prevention"

Prostate cancer is the most common cancer in American men, accounting for 33% of male cancer cases in 2005. Factors influencing prostate cancer risk include age, race, family history, hormone levels, chronic inflammation, and diet. Of these factors, we may be able to influence inflammation, and we can definitely influence our diet. The U.S. has the highest rate of prostate cancer in the world, and Asian countries have the lowest. However, migration studies show that Asian men who have moved to the U.S. have a much higher rate of prostate cancer,

suggesting that lifestyle factors, such as diet, play a significant role in development of prostate cancer (and not just genetics).

One significant dietary nutrient that is limited in the U.S. diet and that can affect prostate cancer risk is zinc. Zinc is vital for the functioning of over 300 enzymes and over 1000 proteins in the human body. The prostate contains the highest concentration of zinc of all soft tissues in males. Studies have shown that lower zinc intake is associated with increased rates of prostate cancer. Through our research, we have shown that zinc deficiency leads to greater oxidative stress, increased DNA damage, decreased DNA repair, and, thus, increased cancer risk.

We are also taking a whole-food approach to dietary cancer chemoprevention. Rather than using individual compounds extracted from foods, we are looking at the effect of whole foods, which provide a combination of nutrients and phytochemicals. This combination possibly provides more protection than one or two compounds alone. For example, a recent study with rats showed that whole tomato powder provided greater protection from death due to prostate cancer than just lycopene (the red compound in tomatoes). We are taking this same approach to study the effects of high-soy diets and tea intake. High soy and tea intake may, in part, explain the lower prostate cancer risk in men living in Asian countries where soy and tea are commonly consumed.

Our recent research demonstrates that whole soy extract has more anti-carcinogenic effects on prostate cancer cells than individual soy isoflavones (genistein and diadzein). This was performed on both early- and late-stage cancer cells in the lab, and whole soy extract proved more effective than isoflavones for both cases.

In other studies, we hormonally induced prostate inflammation in rats. Uncontrolled, long-term prostate inflammation can lead to prostate cancer. We then fed the animals whole soy, green tea, or a combination of soy and green tea. Neither tea nor soy alone showed much effect. However, the combination of soy and tea significantly decreased markers of inflammation and increased levels of an anti-inflammatory protein. This may inhibit hormone-induced inflammation that ultimately contributes to prostate cancer. LPI

Tammy Bray, Ph.D.
Principal Investigator, Linus Pauling Institute
Dean, College of Health and Human Sciences,
Oregon State University

"Antioxidants, inflammation, and diabetes"

In people with diabetes, the body either does not make enough insulin or does not respond to insulin. Insulin's job is to transport sugar from the bloodstream into cells so the cells can make energy. To deal with these abnormally high blood sugar levels, the body gets rid of the sugar via urine. A classic indication of uncontrolled diabetes is frequent urination and uncontrollable thirst as the body rids itself of sugar. There are two major types of diabetes:

Type 1 (T-1) diabetes, formerly known as juvenile-onset diabetes, mainly strikes children and young adults and accounts for 5-10% of all diagnosed cases. T-1 diabetes is caused by the destruction of pancreas cells that make insulin, called beta-cells. T-1 diabetes can develop within weeks or days. The cause of T-1 diabetes is unknown, but it may be related to free radical attack from an acute immune response.

More common is Type 2 (T-2) diabetes, formerly known as adult-onset diabetes, which accounts for 90-95% of all diagnosed cases. Unlike the quick onset as seen in T-1 diabetes, T-2 diabetes is associated with a prolonged (months to years) pre-diabetic period typically associated with obesity and/or lack of exercise. T-2 diabetes is due to the body's development of insulin resistance. This means that the pancreas makes more and more insulin, but the body's insulin receptors no longer react adequately to it.

Our research is currently investigating the ability of antioxidants in delaying the development of T-1 diabetes. We found that several antioxidant compounds (N-acetylcysteine, alpha-phenyl-tert-butylnitrone, and zinc) helped reduce hyperglycemia (elevated blood sugar levels). We then did further research to understand how these antioxidants protect against diabetes development. We found that inflammation is triggered in the pancreas, leading to cell death and decreased insulin production. These findings may help in developing effective preventive or therapeutic strategies for T-1 diabetes.

Diabetes is also associated with delayed wound healing, sometimes leading to amputation. It is believed that wound-healing delays are due to impaired blood flow and low blood oxygen levels caused by high blood sugar levels and inadequate protein and antioxidant intake. Our research shows that prolonged activation of inflammation pathways also contribute to delayed wound healing. Delaying the onset of these complications can help to increase quality of life for people with diabetes.

T-2 diabetes, associated with a prolonged pre-diabetic period, can be addressed with proper diet, weight control, and physical activity. Insulin resistance is significantly reduced through moderate exercise and can help delay or even prevent development of diabetes in obese or inactive people. Proper diet, weight control, and physical activity are also very effective to maintain blood sugar levels even after diabetes has developed. LPI

Maret Traber, Ph.D.
Principal Investigator, Linus Pauling Institute
Professor of Nutrition and Exercise Sciences,
Oregon State University

"Everything and much more than you ever wanted to know about vitamin E"

Vitamin E (tocopherol) is a potent fat-soluble antioxidant that must be consumed from the diet. Although vitamin E is required by humans, we don't know its specific function. We know that when there is an excess of oxidants, damage occurs to DNA, proteins, fats, and carbohydrates within our bodies, which can be partly inhibited by vitamin E. Several forms of vitamin E are made by plants, but only one form is required by the body. The alpha form remains in our blood plasma the longest, about 60 hours compared to less than 15 hours for other forms, like gamma-tocopherol. Given that all forms of vitamin E are antioxidants, understanding how the body chooses alpha-tocopherol has been a major thrust of the Traber laboratory. Our studies have shown that all of the forms are absorbed by the digestive system in the same way that dietary fats are absorbed. Then a liver protein, alpha-tocopherol transfer protein, selects

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alpha-tocopherol for secretion into the blood, while the other forms are metabolized and excreted.

Through daily living, our bodies create reactive oxygen species (ROS). The levels of ROS increase significantly in endurance exercise and can exceed the body's antioxidant systems. We studied whether antioxidants would protect runners participating in an ultramarathon (50 km event) by daily administration of 300 mg of vitamin E and 1000 mg of vitamin C for six weeks before the event. Antioxidant takers compared with placebo takers had lower levels of oxidative stress markers. After the race, women recovered rapidly, while men showed elevated levels of oxidative stress throughout the recovery period. While taking vitamins E and C helped prevent oxidative damage to fats, they did not have other beneficial effects, such as faster recovery or improved inflammatory responses. We also studied vitamin E requirements in cigarette smokers who have 40% higher levels of markers of oxidative damage. Knowing the interaction of vitamin E and oxidative stress, we wanted to see whether smoking increases vitamin E requirements. We found that vitamin E disappears from the plasma about 13% faster in smokers compared to nonsmokers. We also found that higher vitamin C intakes decreased the rate at which vitamin E disappears in smokers. This is the first study in humans showing that vitamin C works to recycle vitamin E.

Our studies show that vitamin E is necessary to protect us from oxidative stress. However, estimates of nutrient intakes showed that 90% of men and 96% of women do not consume at least the estimated average requirement for vitamin E (12 mg). The recommended dietary allowance (RDA) for adults is 15 mg/day (22 IU d-alpha-tocopherol or 33 IU dl-alpha-tocopherol). In order to make sure that you consume adequate amounts of vitamin E, you should choose foods like almonds, sunflower seeds, hazelnuts, spinach, and sunflower and safflower oils. Alternatively, a multivitamin or a vitamin E pill will provide the required amount of vitamin E. Importantly, to increase vitamin E absorption from supplements, it is best to take them with food containing some fat. It is important to consume this level of vitamin E because the latest findings from a large clinical trial demonstrated that people with the highest vitamin E intakes from food had lower risks of all chronic diseases. LPI

Balz Frei, Ph.D.
Endowed Chair and Principal Investigator, Linus Pauling Institute, Professor of Biochemistry and Biophysics, Oregon State University

"How to live longer and feel better – what we have learned in the last 20 years"

2006 marked the 20th anniversary of Linus Pauling's book *How to Live Longer and Feel Better*. This book has been read throughout the world and has affected many people's lives and behaviors, as well as the health research field. Pauling outlines a "Regimen for Better Health" in which he makes recommendations for diet and lifestyle, including supplementation of vitamins in doses often considerably higher than the RDA. (It should be noted that current research does not support some of Pauling's recommendations, such as taking doses of vitamin A above the current Tolerable Upper Intake Level of 10,000 IU per day.) Pauling's book served as an inspiration to many scientists who are studying orthomolecular medicine (the practice of varying concentrations of normal substances in the human body to maintain good health and treat disease), particularly in the field of micronutrients. His book is also very relevant to current health issues like obesity and excessive sugar consumption. The book is intended for the general public and has had a beneficial effect on public education and people's diets and lifestyles.

Pauling took particular interest in vitamin C, and researchers continue to build upon his work. He recommended taking 6-18 grams of vitamin C per day. Current studies show that peak plasma and blood cell concentrations of vitamin C do not increase with oral doses higher than 400 mg. However, these tests were performed on young, healthy people, and older or diseased individuals may have different needs, a concept Pauling termed "biochemical individuality."

Vitamin C has also been shown to be an excellent antioxidant in human plasma. It protects against oxidative stress caused by inflammation, excessive exercise, or tobacco smoking. Oxidative stress impairs our body's ability to relax arteries and regulate blood pressure. Vitamin C may help people with certain types of heart disease and high blood pressure. In a study of 15,000 men aged 45-79 years, higher plasma levels of vitamin C were associated with decreased risk of cardiovascular disease. Risk was decreased by as much as 60% for the group with highest vitamin C levels in their plasma, and this group also consumed the most fruits and vegetables. Another study showed a 30% coronary heart disease risk reduction in women taking vitamin C supplements of 400 mg/day or more for more than ten years.

In his book, Pauling also recommended moderating dietary fat and alcohol consumption. Current studies show that the amount of fat in the diet is less important than the type of fat consumed. Polyunsaturated fats and monounsaturated fats both decrease LDL cholesterol ("bad" cholesterol) and raise HDL cholesterol ("good" cholesterol), thus exerting a beneficial effect on heart disease risk. These unsaturated fats are found, for example, in vegetable oils, flaxseed, salmon, tuna, trout, olive oil, canola oil, and nuts. Saturated fats, such as animal fats (butter and meat) and tropical oils (palm and coconut oils), somewhat raise HDL but also greatly raise LDL cholesterol and, thus, have an overall adverse effect on heart disease risk. The worst fats are the *trans* fats, which are found in hydrogenated oil products like stick margarine and vegetable shortening. Current studies also show that heart disease is reduced by about 30% in men consuming up to two alcoholic drinks per day and women consuming up to one alcoholic drink per day. The form of alcohol (wine, beer, or liquor) does not seem to matter.

Pauling was also researching whether very high doses of vitamin C could benefit terminal cancer patients. For these studies, 10 g/day were given intravenously (IV) and then followed by 10 g/day orally indefinitely (until the patient's death). This vitamin C regimen seemed to increase the survival time and improve quality of life of the cancer patients. Later studies at the Mayo Clinic with only high oral doses of vitamin C proved ineffective, suggesting that for vitamin C to be effective in cancer therapy, plasma levels may have to be boosted by IV infusion. Recent research studies in the lab indicate that very high levels of vitamin C will kill cancer cells but not normal cells by producing certain reactive oxygen species. Similarly high levels of vitamin C can be achieved in humans by IV infusions but not oral supplementation. Because of these findings, a phase I safety trial of intravenous vitamin C in advanced cancer patients is currently under way, with the ultimate goal to revisit Pauling's thesis that IV vitamin C could benefit cancer patients. LPI

Developments

Scott Palmer
LPI Director of Development

Dr. Tory Hagen has an implacable foe: aging. He and the other members of his lab are looking at the role that "age-essential" micronutrients play in helping people live longer, healthier lives. The Institute believes that an optimum diet is critical to achieving maximum healthspan.

Tory is an LPI principal investigator (PI) working in *Aging and Neurodegenerative Diseases*, one of three major core areas of research here at the Institute. The other two core research areas are *Cardiovascular and Metabolic Diseases* and *Cancer Chemoprotection and Therapy*. Currently the Institute has ten PIs—four in *Cancer Chemoprotection and Therapy*, four in *Cardiovascular and Metabolic Diseases*, and two in *Healthy Aging and Neurodegenerative Diseases*.

Our long-term goal is to have five PIs in each of our three core areas of research. Over the next five years we hope to add three investigators in *Aging and Neurodegenerative Diseases* and one each in *Cardiovascular and Metabolic Diseases* and *Cancer Chemoprotection and Therapy*.

One of our first priorities for expansion is in *Aging and Neurodegenerative Diseases*, with a particular focus on healthspan extension. Adding an additional PI to work with Tory is a key element in our effort to expand our research into aging, but we face some unique challenges.

Part of the reason for the Institute's success has been a focus on collaboration and quality. Bringing top scientists from different disciplines together and creating an environment that strongly encourages an interchange of ideas and concepts has been one of the hallmarks of the Linus Pauling Institute.

As we continue to grow there are some important issues that we need to address. From where are we going to recruit our new PIs? How much will it cost to recruit them, and where will we get the money? And where will we put them when they get here?

The answer to the first question is relatively easy. The Linus Pauling Institute has an enviable international reputation within the scientific community. The quality of our research, the collaborative environment, and the academic independence PIs enjoy at the Institute and Oregon State University (OSU) has always been a strong magnet for top scientists.

The answer to the second question is more problematic. We often find ourselves competing with other leading research universities for the best and brightest scientists. For a top scientist, the "start-up package" that a research institute offers is an important part of the decision-making process as to where they will go. Start-up costs include computers, office and lab furniture, scientific instruments, supplies, staff support, and relocation expenses. Not surprisingly, there is a direct relationship between the sophistication and complexity of the research being conducted and the cost of the equipment.

The typical start-up package a highly recruited scientist is offered at a more well-known university is generally higher than OSU is able to offer. That's where the support of our many friends plays a crucial role for us. While we will likely never be able to match dollar for dollar top-end recruitment packages, the unrestricted donations and gifts we receive from people like you enable us to offer competitive start-up packages. The Institute has used, and will continue to use, donated funds to help attract and recruit the best scientists.

The third question is the most difficult to answer and will likely determine the future growth of the Institute more than any other issue. When Dr. Linus Pauling founded the Institute in 1973 he envisioned a physical environment where the different PIs would be working in relatively close proximity. That way a biophysicist in one lab could easily collaborate with a chemist in another lab to find an answer to a problem that neither one could solve by themselves.

Due to the Institute's ability to secure the necessary funding and then recruit outstanding PIs over the past ten years, we now have labs and scientists spread out all over the OSU campus. Now, one of the unfortunate realities that we face is that the only time that all of our scientists and researchers are together at one time in a collaborative environment is during the annual LPI scientific retreat that is held every August.

Tory's lab is located on the first floor of a fifty-year-old building. Quality research space at OSU is at a premium and when we are able to recruit another PI to work with Tory on healthspan extension, there is no guarantee that scientist and their lab would even be in the same building as Tory, let alone on the same floor.

The good news is that the leadership of OSU is very supportive of the Linus Pauling Institute. The University is working closely with the OSU Foundation to develop a permanent long-term solution to the Institute's future growth needs. If successful, Tory will be working side-by-side with other PIs in state-of-the-art research space—an objective that will benefit anyone who is interested in aging research.

The Power of Planned Giving

This past year, the Linus Pauling Institute passed two important milestones. On November 1, 2006, we celebrated ten years here at Oregon State University. A few weeks after our celebration, we were notified that the Institute was the beneficiary of two large, unexpected bequests. Since 1996, including these two latest gifts, the Linus Pauling Institute has received more than $14 million in bequests and planned gifts from friends who included the Institute in their will or estate plan.

Quite simply, the Institute would not be in existence today without the amazing generosity and foresight of all of those who have provided for the Institute in their will or estate plan. Unlike research into new medical treatments, very little of what we do has the potential to be patented and then licensed to a commercial company. As a result there is no incentive for companies to fund innovative, new research in the field of orthomolecular medicine. Without the strong, ongoing philanthropic support of you and our other donors, the work of the Institute would come to a halt.

Your gift does make a difference. For our donors and supporters who will be 70½ years old this year, there is a new, easy way for you to support the Institute. The Pension Protection Act of 2006 allows individuals aged 70½ or older to make gifts up to $100,000 to qualified charitable organizations like ours using funds transferred directly from their IRAs. Someone who does give money from their IRA does not have to pay taxes on the amounts transferred.

For more information on how to give to the Linus Pauling Institute from your IRA, or how to include the Institute in your will or estate plan, please contact me at (541) 231-6751, or by email at scott.palmer@oregonstate.edu. LPI

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.

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DIET AND OPTIMUM HEALTH

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CONFERENCE TOPICS

Health promotion and disease prevention by lifestyle and diet, including vitamins, minerals, and phytochemicals; and the role of oxidative stress and antioxidants in human health and disease

Look for these informative articles inside!

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

Micronutrient Research for Optimum Health

Linus Pauling Institute

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