

OSU Oregon State University



# The Linus Pauling Institute

R E S E A R C H N E W S L E T T E R



## From the Director

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

The Linus Pauling Institute continues to expand its Healthy Aging Program with the recent recruitment of Dr. Adrian “Fritz” Gombart, who joined us in September from the Cedars-Sinai Medical Center and the David Geffen School of Medicine at the University of California, Los Angeles. Dr. Gombart holds B.S. and M.S. degrees in Biology and Genetics, respectively, from Oregon State University, and a Ph.D. in Microbiology from the University of Washington in Seattle. Dr. Gombart will work closely with Dr. Tory Hagen, director of LPI’s Healthy Aging Program and recently endowed Jamieson Chair in Healthspan Research. Dr. Gombart’s research program focuses on understanding the role of vitamin D in innate immunity and immunosenescence. He also has done work on vitamin A and has a general interest in the biological mechanisms affecting the immune system and its decline during aging. On behalf of all of us at LPI, a warm welcome to Dr. Gombart and his family!

We are also getting ready for our fifth LPI conference on “Diet and Optimum Health,” which will be held in May 2009 in Portland, Oregon. As in previous years, the conference will emphasize dietary and lifestyle approaches to improving human health and preventing or treating disease, including dietary supplements, micronutrients, and phytochemicals; and the role of oxidative stress and dietary antioxidants in human health and disease. In addition to the LPI Prize for Health Research and a public session by Dr. Marion Nestle from New York University on “What to eat?,” the program features scientific sessions on metabolic syndrome, healthy aging, immune function, epigenetics, and vitamin K. A detailed program can be found on pages 8-9 in this Newsletter.

Finally, two intriguing scientific articles were published recently, and picked up by the popular press, on the role

*continued on page 2*



## Zinc: From Diabetes to Cancer

*An interview with Emily Ho, Ph.D.  
Associate Professor of Nutrition  
and Exercise Sciences  
LPI Principal Investigator*

**Q.** *How did you decide on a career in nutritional science?*

**A.** At the University of Guelph in Ontario, Canada, I majored in biochemistry and biology. One of my first lab experiences was in a free radical lab with someone working on the antioxidant enzyme superoxide dismutase and Lou Gehrig’s disease. I worked in John Phillips’s lab, and one of his collaborators was Tammy Bray, who was in the nutrition department and is now a Dean at Oregon State University and a member of LPI. She was also really interested in free radical metabolism and more of an applied researcher rather than a geneticist. I really liked the research and free radical theory of disease, so I started to lean towards how nutrients can really make a big impact on disease. I did an undergraduate project with Tammy and switched over to the nutrition program after my sophomore year.

**Q.** *Your early work was on diabetes. What is the difference between type 1 and type 2 diabetes?*

**A.** The common name for type 1 diabetes is juvenile onset diabetes, but that’s changing now that type 2 diabetes is affecting juveniles. In type 1 diabetes, there is an immune destruction of the insulin-producing beta cells in the pancreas, so these people can no longer produce insulin. Type 2 diabetes is more of an insulin-resistance problem. These people can still produce insulin, but it doesn’t function to respond to elevated blood glucose anymore. So the two are very different even though both result in high blood glucose that causes problems like heart disease and renal failure.

**Q.** *What causes the destruction of beta cells in juvenile diabetes?*

**A.** That’s the golden question. It’s unknown what triggers the immune attack on the beta cells. Years ago we weren’t really interested in how that happens as much as

*continued on page 3*

— DESIGNATED BY THE NATIONAL INSTITUTES OF HEALTH AS A —

**Center of Excellence for Research on Complementary and Alternative Medicine**

of vitamin C in cancer therapy, a topic of great interest to us. The first was a paper in the prestigious journal, *Proceedings of the National Academy of Sciences USA (PNAS)*, by the group of Dr. Mark Levine at the National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health. They showed that intraperitoneal injection (into the abdominal cavity) of large, “pharmacologic doses” of vitamin C into mice decreased by about half the growth and weight of human, rat, and mouse tumor xenografts (implanted cancer cells growing into tumors). This work followed a number of publications by the same group showing that millimolar concentrations of extracellular vitamin C in the fluid surrounding cells and tissues kill cancer cells but not normal cells by a pro-oxidant mechanism, involving the production of hydrogen peroxide by vitamin C and a yet-to-be-identified metalloprotein. Interestingly, such millimolar concentrations of vitamin C can be achieved in humans only by intravenous infusion, not by diet or oral vitamin C supplementation. This finding may explain the discrepant results by Drs. Linus Pauling and Ewan Cameron in the 1970s, showing that intravenous vitamin C is of substantial benefit in terminal cancer patients, and the “repeat” of those studies at the Mayo Clinic published in 1979 and 1985, which used only oral vitamin C and did not find a benefit in cancer patients. The Editor-in-Chief of *PNAS* invited me to write a commentary on Dr. Levine’s paper, in which Stephen Lawson and I concluded that the new data provide a compelling rationale for revisiting the still-controversial issue of vitamin C and cancer and that the potential anticancer value of vitamin C should be vigorously

pursued in randomized, double-blind, placebo-controlled clinical trials of high-dose intravenous vitamin C.

The second study was published in a leading cancer journal, *Cancer Research*, by Dr. Mark Heaney’s group at the Memorial Sloan-Kettering Cancer Center in New York City. The paper carried the rather alarmist title “Vitamin C antagonizes the cytotoxic effects of antineoplastic drugs.” A closer look at this paper reveals that they did not use vitamin C in any of their experiments but instead used its oxidized form called dehydroascorbic acid (DHA). Dehydroascorbic acid is not available as a dietary supplement, and any DHA formed in the human body by oxidation of vitamin C is quickly disposed of by known chemical and biological mechanisms. What’s more, the studies by Heaney and colleagues were performed on two cancer cell lines in Petri dishes, and the single study in animals used DHA doses known to be toxic. Under these conditions, the authors found that DHA may very modestly lower the effectiveness of the anticancer drug doxorubicin. In sharp contrast, in reviewing the current scientific evidence from human studies, other researchers have concluded that the relationship between vitamin C and chemotherapy is promising in terms of increased survival times and decreased toxicity (adverse side effects) of chemotherapeutic drugs. They cautioned, however, that the interaction between vitamin C and chemotherapy or radiation therapy should be further addressed in large, well-designed, clinical studies before any conclusions can be drawn. It is likely that vitamin C, by itself or in combination with standard therapy, will show substantial benefit in the treatment of certain types of cancer. **LPI**



Dr. Gombart and his family

## Dr. Adrian “Fritz” Gombart joins LPI

Dr. Fritz Gombart has joined LPI’s Healthy Aging Program as a new principal investigator.

He also holds the position of associate professor in OSU’s Department of Biochemistry and Biophysics.

Since 1993 Dr. Gombart has worked as a research scientist at Cedars-Sinai Medical Center in Los Angeles, initially in the Division of Hematology/Oncology and then in the Department of Biomedical Sciences.

Dr. Gombart has focused on the role of vitamin D in the human immune system, especially concerning microbial infection and cancer.

Humans are born with an “innate” immune system that defends us against pathogens like bacteria and viruses. It is a complex system comprised of mast cells; phagocytes, consisting

of neutrophils, macrophages, and dendritic cells; natural killer cells; eosinophils; and basophils, which release histamine.

The “adaptive” immune system is responsible for long-term immunity and is comprised of lymphocytes called B cells and T cells. B cells manufacture antibodies against antigens, and T cells help mediate the overall immune response.

Neutrophils and macrophages of the innate immune system respond to cytokines—inflammatory molecules released at the site of infection—and migrate to the infection where they engulf pathogens and kill them by releasing reactive oxygen species. Neutrophils, mast cells, and natural killer cells can also kill pathogens by secreting cathelicidin, an antimicrobial protein. Since bacteria do not easily develop resistance to this protein, scientists have been interested in enhancing its production by immune cells during infections. Using cultured human cells, Dr. Gombart and his colleagues have shown that the biologically active form of vitamin D—1,25-dihydroxy-vitamin D<sub>3</sub>—induces the expression of the gene encoding cathelicidin, thereby increasing the amount of this antimicrobial protein. Further development of this observation may provide an orthomolecular strategy for augmenting the immune response to pathogens, which is especially important as we age. **LPI**

in how the immune response could be mitigated. Antioxidant supplementation was one strategy to help stop some of the consequences of the immune attack on the beta cells.

**Q. Is that because reactive oxygen species are implicated in the destruction of these cells?**

**A.** Exactly. Immune cells produce reactive oxygen species to kill pathogens like viruses. In type 1 diabetes, the immune system is activated, and macrophages, monocytes, and neutrophils start generating lots of oxidants to try to destroy the pathogen, and the beta cells are unfortunate victims of the attack.

**Q. Why are beta cells vulnerable to these oxidants early in life but not in adults?**

**A.** That's unknown. The islet cells are uniquely sensitive to oxidative stress, especially early in life. For example, if a premature infant is put into a high oxygen environment because his or her lungs are not developed, lots of oxidants are produced and pancreatitis may occur. The pancreas becomes inflamed because it doesn't yet have its full complement of antioxidant defense mechanisms in place.

**Q. And that can result in the onset of juvenile diabetes?**

**A.** Well, the association has not been fully established, but it is a working hypothesis. Unlike the lungs, the pancreas is susceptible to oxidative stress, probably because its protection by antioxidant enzymes and micronutrients has not fully developed.

**Q. Has that been studied in animals?**

**A.** Certain chemicals that generate reactive oxygen species can selectively destroy the beta cells in rodents. A specially bred mouse develops type 1 diabetes very similar to humans through an autoimmune attack that's age dependent.

**Q. What dietary strategies might be important in influencing the risk for type 1 diabetes?**

**A.** We study zinc, largely because zinc is an antioxidant and also plays a critical role in insulin storage. Insulin is stored as a zinc crystal inside the islet cells in the pancreas, so it has an important function to preserve insulin function. Type 1 diabetes has a genetic component, but we know from studies of twins that it's not nearly as strong as some environmental factors.

**Q. Has anyone ever examined antioxidant status in youngsters to find out if it is associated with protection against the development of type 1 diabetes?**

**A.** People have tried, but it's a really difficult question. Diabetes itself causes high glucose that generates a lot of oxidative stress. By the time a child is diagnosed, more than 90% of their beta cells have already been destroyed.

**Q. You published a study showing that N-acetylcysteine inhibits the activation of inflammatory molecules in mice. What is that compound?**

**A.** We used it as a pro-drug to produce glutathione in the mice. Glutathione is a very important thiol-based antioxidant in the body, but if you take it as a supplement,

it will get destroyed in the digestive system. Our strategy was to provide a precursor to glutathione so that more of it will be made in the body.

**Q. Does N-acetylcysteine have any toxicity?**

**A.** As far as I know, no significant side effects or toxicity have been reported.

**Q. How does glutathione affect inflammation?**

**A.** NF- $\kappa$ B is a transcription factor that turns on a lot of genes involved in the immune response, acting as a master regulator of immune response. NF- $\kappa$ B is redox sensitive—oxidative stress can activate it, which causes the expression of genes that cause inflammation. That, in turn, produces more oxidative stress that further amplifies the process. Glutathione or other antioxidants may help break that cycle by stopping the oxidative stress, attenuating NF- $\kappa$ B activation, and breaking the endless cycle of self-propagating chronic inflammation. We think that zinc also inhibits NF- $\kappa$ B activation.

**Q. How might zinc protect against diabetes?**

**A.** Zinc plays a big role in helping to maintain a healthy immune system. It also acts as an antioxidant and stops some of the proinflammatory effect of NF- $\kappa$ B.

**Q. Are diabetics zinc deficient?**

**A.** Not many studies have examined zinc status in diabetics. The main problem is that zinc status is very difficult to assess—there is really no good biomarker for marginal zinc deficiency, which is what we have in the United States. In developing countries where there is a lot of severe zinc deficiency, there also is an increased incidence of chronic infections.

**Q. In some countries people consume a lot of phytic acid from cereals and grains that binds to zinc, making it biologically unavailable. Is diabetes common in those areas?**

**A.** Diabetes is not a very significant problem for countries like Iran and Bangladesh, where phytic acid-containing foods are commonly consumed. In those countries gastrointestinal and respiratory infections are some of the major causes of death, so they are more intensely studied. Also, in many of those countries caloric intake is low compared to Western countries.

**Q. How does zinc affect DNA damage and repair?**

**A.** Zinc is a really interesting nutrient because it does such a wide variety of things. Its antioxidant function may help protect from oxidative damage associated with cancer risk. Zinc is also a part of many different metalloproteins, some of which are involved in DNA repair and replication, so when you don't have adequate zinc, a lot of these enzymes might not function properly and the ability to repair DNA is impaired.

**Q. Do most people get enough zinc in their daily diet?**

**A.** Most people get enough zinc. However, according to the latest NHANES data, which is a huge database of dietary

*continued on page 4*

intake patterns in the United States, up to 12% of the U.S. population is not getting the recommended dietary intake of zinc. In the elderly, the number is closer to 50%. So, in older individuals, zinc deficiency is an especially important problem.

**Q.** *The RDA of zinc for adult men is 11 mg/day and for adult women, 8 mg/day. How are those RDAs determined, and why is there a difference in the RDA for men and women?*

**A.** The way that the RDAs are determined is a long story. There is a lot of debate about whether those values are actually high enough. Zinc is a really tough nutrient to study in humans. We try to establish the RDAs based on human data from zinc balance studies. Zinc can be radiolabeled in order to trace it, and then we can see how much stays in the body and how much is excreted. If you have sufficient zinc, more will be excreted, but if you have low zinc status, more will be retained. Some animal models are used, but it's difficult to extrapolate to humans. The main reason why there are differences between men and women may be average body size, which, of course, has changed appreciably in recent years.



Dr. Emily Ho and her son Ryan

**Q.** *The tolerable upper level of intake (UL) for zinc is 40 mg/day for both men and women. Do you think that's reasonable?*

**A.** I think that is reasonable. Zinc itself is actually very non-toxic. The main problem with zinc is that it competes with other minerals. So the UL for zinc is based on its effect on copper absorption. There is a chance that you might compromise copper status if you exceed the UL for zinc.

**Q.** *It has been suggested that too much iron or copper may be detrimental to health because it accumulates, especially in post-menopausal women and in men. Do we need to worry about zinc accumulation with age?*

**A.** No. Unlike iron and copper, which can be pro-oxidant and cause oxidative stress at high concentrations, zinc isn't detrimentally reactive. Again, the main problem with excess zinc is interference with other metal nutrients. There is also little ability to store zinc in the body.

**Q.** *You mentioned that zinc has indirect antioxidant function, probably as a component of copper-zinc superoxide dismutase and in other ways. How do you study this?*

**A.** We're using cell cultures, rodents, and some human subjects to check how zinc intake affects total antioxidant capacity and oxidative stress. We do that by measuring a biomarker of oxidative stress called F<sub>2</sub>-isoprostanes, which are formed from the oxidation of arachidonic acid, an omega-6 fatty acid.

**Q.** *Are there any symptoms of marginal zinc deficiency?*

**A.** Yes, there are a lot of symptoms of marginal zinc deficiency. Zinc is involved in so many processes that symptoms of marginal deficiency are pretty non-descript, unlike vitamin C, where classic deficiency symptoms are clear. With zinc, so many different proteins can be affected, and you see very generalized deficits in immune function, slow growth and general malaise in children, or gastrointestinal problems.

**Q.** *Do you think that zinc lozenges may be helpful in treating the common cold?*

**A.** I think that the evidence is somewhat equivocal. I doubt that there is a strong, direct antiviral effect, but zinc does affect immune function. It's possible that marginal zinc deficiencies are going relatively undetected in the U.S. When you take the zinc supplements, you start to restore your zinc status, and that helps to boost your immune system. Some research has used zinc sprays, but one of its side effects is a loss of smell, sometimes permanently.

**Q.** *You have done a lot of work with zinc and prostate cancer using cell cultures. What have you found?*

**A.** The prostate has the highest concentration of zinc of all the soft tissues in the body—bone is the only other tissue that has more zinc. The prostate accumulates zinc, but we don't know why. In prostate cancer, the zinc levels dramatically drop dose-dependently with disease progression. We want to understand these phenomena better.

**Q.** *Do zinc levels decline with age in men?*

**A.** We're looking into that. As I mentioned, zinc status in humans is really difficult to assess, but there is some evidence that zinc levels decrease with age. We think that absorption may change with age. Older people eat less, and they are not able to absorb as much zinc. Most of the studies have been done in women. If you give zinc supplements to young women and older women, plasma zinc levels will increase in the young but not in the older women. With men, it doesn't

look like zinc supplementation is going to be a cure for prostate cancer. We haven't seen much effect of zinc in decreasing cancer in our rodent studies, but we have found that low zinc levels cause problems in the prostate with respect to oxidative stress, DNA damage, and mutations that could increase the risk for cancer.

**Q. Does zinc have any effect on prostate cancer malignancy?**

**A.** The more aggressive cancers seem to have less zinc. One problem is that the cancer cells have adapted to a low zinc status, so if you give zinc, they are more resistant. We have been a little bit disappointed with the fact that once you have cancer, zinc doesn't help much. On the other hand, zinc may play an important role in preventing prostate cancer.

**Q. What are the most effective dietary strategies to protect against prostate cancer?**

**A.** There's no magic bullet, but consumption of fruits and vegetables is associated with decreased risk. Our research suggests that cruciferous vegetables might be helpful. People in Asia have a very low incidence of prostate cancer, but when they move to the United States, it dramatically increases because of dietary changes. Also, there has been a low prostate cancer risk in China, but as they become more industrialized, there is a slow but steady increase in prostate cancer. Eating a lot of fat or red meat seems to increase the risk. Obesity is a risk factor for prostate cancer partly because of its associated inflammation and hormones that may change with increased adiposity.

**Q. You found that elevated estrogen levels in men who have high testosterone levels are associated with an increased risk of prostate cancer. Why?**

**A.** The diet may affect that. For example, soy compounds can affect estrogen levels. It seems to be the ratio that's important, not necessarily the absolute amount. The higher estrogen ratio is more pro-inflammatory in men.

**Q. Are you interested in a whole food approach rather than supplements?**

**A.** Yes. The substances in supplements may not be absorbed as well as those in food. And foods, like tomatoes, have not only lycopene, but vitamin E and some other polyphenolic compounds that act synergistically and target different cellular pathways. In some studies, a lycopene supplement was not as effective as freeze-dried tomato in preventing cancer.

**Q. Your recent studies have examined the role of sulforaphane in tumor suppression. What is sulforaphane, and how does it work?**

**A.** Sulforaphane is a chemical found in plants, specifically in cruciferous vegetables like broccoli, cauliflower, and cabbage. It's an isothiocyanate and works through multiple mechanisms. Paul Talalay's group at Johns Hopkins did a lot of the groundbreaking work with sulforaphane. It helps get rid of carcinogens through a detoxification mechanism in the early stages of

carcinogenesis. The problem is that you don't usually know when you were exposed to possible carcinogens, so the timing of exposure and sulforaphane ingestion is problematic. By the time of cancer diagnosis, initiation is an historical event. There is interest now that sulforaphane may act in the post-initiation phase—not just getting rid of carcinogens but actually stopping some of the uncontrolled proliferation of cells. It may stop the proliferation by affecting epigenetics—a process where the environment affects gene expression without changing the DNA code.

**Q. Specifically, you found that sulforaphane inhibits histone deacetylase. What is that process, and why would it protect against cancer?**

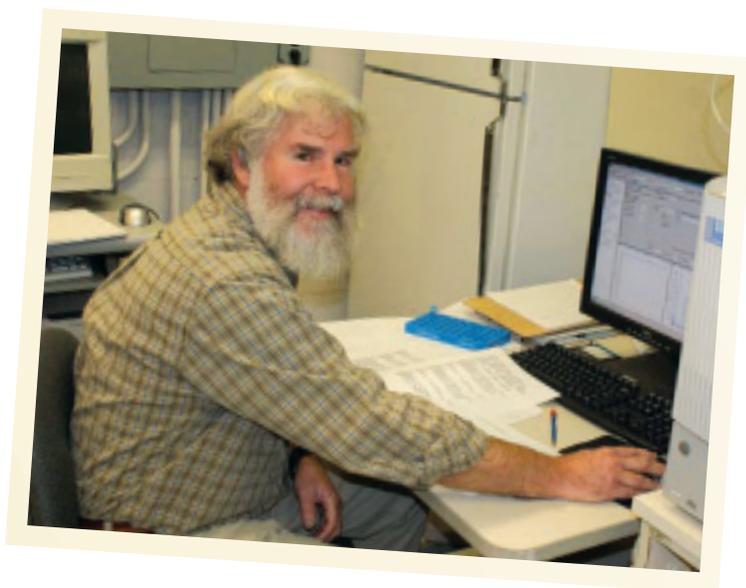
**A.** Histones are proteins around which DNA winds. When histones are acetylated, genes are turned on. When histone deacetylases remove the acetyl groups, genes are turned off. In cancer, histone deacetylases that remove the histone acetylation group are overactive. So genes that are normally active to help combat cancer, like tumor suppressor genes, are turned off. Histone deacetylase inhibitors like sulforaphane reverse this process so that tumor suppressor genes can be turned on.

**Q. Has sulforaphane been tested in humans?**

**A.** This is very much in the early stages. We start out with cell cultures and animal models. Both our group and the Talalay group have started to look at sulforaphane in people, checking safety, toxicity, and metabolism.

**Q. Would the amount of sulforaphane in vegetables that we consume be effective, or do you think that supplements may be necessary?**

**A.** The current recommendation is to consume five to nine servings of fruits and vegetables per day, but the amount of sulforaphane in those vegetables may not be sufficient to get the desired effect. **LPI**





## Comparisons of Serum Micronutrients between Okinawan and Oregonian Elderly

Hiroko H. Dodge, Ph.D.  
Assistant Professor  
OSU Department of Public Health

The recent news published by the Alzheimer's Association is gloomy:

"Someone develops Alzheimer's disease every 72 seconds."

"In 2007 there are more than 5 million people in the United States living with Alzheimer's disease."

"Without a cure or effective treatments to delay the onset or progression of the Alzheimer's, the prevalence could soar to 7.7 million people with the disease by 2030."

Alzheimer's disease (AD) is the most prevalent type of dementia. Although there is no cure, modification of lifestyle factors (e.g., diet high in antioxidant nutrients and enriched, stimulating environment) may play an important role in preventing or postponing AD onset and progression. Dementia is an age-associated disease, which means the risk of developing dementia increases as we get older. The fastest growing segment of the population in the United States and most other developed countries is that aged 85 and older (so called "the oldest old"). Unfortunately, this group has a high risk of getting dementia because old age itself is an important risk factor.

We are interested in whether diet and lifestyle explain differences in dementia prevalence. Therefore, we chose to compare Americans and Okinawans. Okinawa is the most southern island of Japan. The island is well known for its high prevalence of centenarians, and past studies have shown that Okinawan elderly not only live longer, but also survive with better physical and cognitive health. Table 1 shows life expectancy for Americans and Okinawans at birth, age 65, and age 85.

Table 1. Life Expectancy at Various Ages (Year 2000)

	At Birth	Age 65	Age 85
<b>MEN</b>			
Okinawa	77.64	18.45	6.85
Japan (overall)	77.72	17.54	5.76
U.S.	74.10	16.30	5.60
<b>WOMEN</b>			
Okinawa	86.01	24.10	8.96
Japan (overall)	84.60	22.42	7.61
U.S.	79.50	19.20	6.70

Sources: [www.mhlw.go.jp/toukei/index.html](http://www.mhlw.go.jp/toukei/index.html) and [www.cdc.gov/nchs/products/pubs/pubd/11ftbl/life/1966.htm](http://www.cdc.gov/nchs/products/pubs/pubd/11ftbl/life/1966.htm)

At age 85, Okinawan women live 9.0 more years on average, while U.S. elderly women live 6.7 more years. Two years difference in life expectancy at this old age is a very big

difference. Table 2 shows the prevalence of dementia in the elderly in the U.S. and Okinawa. The Okinawan elderly seem to have a lower prevalence of dementia. One important factor contributing to their health suggested by the Okinawa Centenarian Study is their low total caloric and saturated fat intake, along with a diet rich in fruits and vegetables.

Table 2. Prevalence of Dementia in the U.S. and Okinawa (%)

	Age 71-79	Age 80-89
<b>TOTAL</b>		
U.S.	4.97	24.19
Okinawa	3.67	14.93
<b>MEN</b>		
U.S.	5.25	17.68
Okinawa	4.39	15.00
<b>WOMEN</b>		
U.S.	4.76	27.84
Okinawa	3.23	14.89

Sources: *Neuroepidemiology* 29:125-132, 2007 and *International Journal of Epidemiology* 24(2):373-380, 1995

A pilot grant from the Linus Pauling Institute allowed us to examine whether Okinawans have higher amounts of antioxidant nutrients in their blood, possibly prolonging their healthy lifespan and helping maintain cognitive function.

We recruited participants in Oregon and Okinawa who were age 85 and older. They were functionally independent and had no major diseases that require visiting a doctor once per month or more. They also had normal cognitive functions, an absence of significant depressive symptoms, and sufficient vision and hearing to complete neuropsychological testing.

For the Oregon sample, we used the stored serum samples of 135 subjects who participated in a *Ginkgo biloba* clinical trial between 2000 and 2001 (published in *Neurology* in 2008). We analyzed the blood sample collected at the baseline before the subjects received *Ginkgo biloba* and vitamin E supplements. For the Okinawan sample, we enrolled 62 subjects who met the inclusion criteria listed above.

Among the relatively healthy elderly participants age 85 and older, the Oregon group contained over 56% obese (BMI>25) and 31% with high triglycerides. The prevalence of these factors was significantly higher than among the Okinawan elderly, of whom about 30% were obese and 13% had high triglycerides. Both groups had a high proportion with hypertension (85% and 87% for Okinawan and Oregon groups, respectively). Metabolic syndrome is characterized by abdominal obesity, elevated triglycerides, high blood pressure, and other factors. People with this condition have an increased risk for heart disease and diabetes, and some studies have shown an association between metabolic syndrome and AD risk, especially among those with atherosclerosis. Therefore, our results among the Oregonians raise some concerns regarding their risk of getting AD in the future. On the other hand, Okinawan elderly had a higher proportion of those with high sodium. It is well known that the Japanese diet is low in fat but high in salt, which contributes to hypertension and resultant cerebrovascular diseases. For example, in 2005, 6.6% of total deaths in the U.S. were from cerebrovascular diseases, while the rate in Japan was 12.3%, almost twice as high as the U.S. rate. On the other hand,

28.9% of total deaths in the U.S. were due to heart disease, but in Japan it was only 16.0%.

At the beginning of the study, the concentration of alpha-tocopherol (vitamin E) in the blood from Okinawans and Oregonians was nearly identical, but blood samples from Oregonians had more than twice the concentration of gamma-tocopherol, which is the most common form of vitamin E in the American diet.

Folate levels in blood are three times higher in the U.S. subjects than in Okinawans. Since 1998, it has been mandatory

in the U.S. to fortify cereals and flour with folate. Possibly because of this high folate intake, Oregonians' homocysteine levels were slightly lower than those in Okinawa, but the difference did not reach statistical significance.

We are going to follow the same participants in Okinawa for two more years. We plan to examine whether the micronutrient values collected at baseline will predict cognitive well-being and/or survival longitudinally. We will keep you updated on what we find in the future.

LPI



## Micronutrients for Older Adults

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

**N**utritional needs change throughout the various stages of life. While overall caloric needs tend to decrease with age, the requirements for individual micronutrients (vitamins and nutritionally-essential minerals) do not decrease. In fact, the needs for some micronutrients, such as calcium and vitamin D, actually increase with age: adults older than 50 years require higher intakes of these two micronutrients. Older adults may also need more dietary antioxidants, such as vitamins C and E, as well as certain B vitamins, including vitamin B<sub>6</sub>, folate, and vitamin B<sub>12</sub>. Micronutrient deficiencies are quite common in the U.S. and other countries, and older adults are especially vulnerable. Since physical activity levels generally decline with increasing age, older adults have lower energy requirements than younger adults. Therefore, it is particularly important for older adults to choose nutrient-rich foods and take a daily multivitamin-mineral supplement. Adequate intake of micronutrients not only ensures that current metabolic needs are met but also may reduce one's risk for chronic diseases, including heart disease, cancer, and osteoporosis, that are more common in older adults.

Micronutrient deficiencies in older adults are most likely caused by a combination of factors, including poor dietary intake and physiological changes that accompany aging. The more common micronutrient deficiencies in older adults are grouped into functional groups; their importance in the body is discussed below.

### Micronutrients important in bone metabolism

Compared to younger adults, adults older than 50 years require higher amounts of two micronutrients important in bone metabolism, calcium and vitamin D. Intestinal absorption of calcium declines with age, as does the capacity to endogenously synthesize vitamin D in the skin. Intestinal absorption of the mineral magnesium may also slightly decline with age, and the recommended intake is higher in adults older than 30 years. All three micronutrients are essential for bone health.

### Calcium

Calcium is the most common mineral in the human body, with about 99% of total body calcium residing in bones and teeth; the remaining 1% is found in blood and soft tissues. It is extremely important that people meet dietary intake recommendations for calcium. Otherwise, the body will demineralize bone in order to maintain blood levels of calcium within a narrow range, which is essential for normal physiological function. Bone demineralization can lead to bone fragility, osteoporosis, and bone fracture. To minimize deleterious effects to bone, older men and postmenopausal women should consume a total (diet plus supplements) of 1,200 mg/day of calcium. Total intake of calcium should not exceed 2,500 mg daily.

### Vitamin D

Adequate vitamin D nutrition is required for optimal calcium absorption, normal bone growth, and maintenance of bone density. Vitamin D also has important roles in maintaining muscle strength. Thus, inadequate vitamin D status results in loss of bone integrity and muscle weakness; both can potentially increase the likelihood of falls and bone fractures in older adults. Clinical trials examining the risk of osteoporotic fracture in older adults have generally found that vitamin D supplementation can slow losses in bone density and decrease the incidence of bone fracture. Some studies suggest that the supplemental threshold for any benefit on bone integrity is 700 IU (17.5 mcg) daily.

Because vitamin D is found in few foods, sun exposure is the main source for vitamin D. However, older adults have a reduced capacity to synthesize vitamin D in skin upon exposure to ultraviolet-B radiation. Older adults also have impaired conversion of vitamin D to its active form in the kidneys. Thus, supplemental vitamin D is especially important for older adults. Emerging evidence suggests that a daily supplemental intake of 2,000 IU of vitamin D may help protect older adults from breast, ovarian, and colon cancers.

### Magnesium

The majority of U.S. adults, regardless of age, do not meet the recommended dietary allowance (RDA) for magnesium. Elderly adults are especially at risk for magnesium deficiency due to relatively low dietary intakes,

*continued on page 9*

# DIET AND OPTIMUM HEALTH

A CONFERENCE ORGANIZED BY THE LINUS PAULING INSTITUTE  
CO-SPONSORED BY THE OXYGEN CLUB OF CALIFORNIA AND OREGON HEALTH & SCIENCE UNIVERSITY

MAY 13-16, 2009 • HILTON HOTEL, PORTLAND, OREGON

## 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

### Wednesday, May 13, 2009

- 2:00 PM Registration begins  
3:30 - 5:15 Oral Poster Presentations  
5:30 - 7:00 Young Investigator Finalist Presentations  
*Chairs: Gayle Orner and Sharon Krueger, Linus Pauling Institute, Oregon State University*  
7:00 Welcome Reception

### Thursday, May 14, 2009

- 6:00 AM Organized Walk/Run  
7:30 Breakfast  
8:15 Welcome and Introduction by Conference Co-Chairs, Balz Frei and Rod Dashwood

#### HEALTHY AGING: NEUROCOGNITIVE DISORDERS AND MOLECULAR MECHANISMS

*Chairs: Tory Hagen, Linus Pauling Institute, Oregon State University and Kathy Magnusson, Department of Biomedical Sciences, Oregon State University*

- 8:30 - 9:00 Healthspan Extension by Resveratrol  
*Kevin Pearson, University of Kentucky*  
9:00 - 9:30 A Low Dose of Dietary Resveratrol Partially Mimics Caloric Restriction and Retards Aging Parameters in Mice  
*Tom Prolla, University of Wisconsin*  
9:30 - 10:00 Epigenetics, Diet, and Aging  
*Craig Cooney, University of Arkansas for Medical Sciences*  
10:00 - 10:30 Coffee/Tea Break  
10:30 - 11:00 Alpha-lipoic Acid and Other Micronutrients in Cognitive Dysfunction  
*Carl Cotman, University of California-Irvine*  
11:00 - 11:30 Dietary Supplements Improve Cognitive Function  
*David Kennedy, Northumbria University, UK*  
11:30 - 12:00 PM Oxidative Stress and Neurodegeneration in Alzheimer's Disease  
*D.Allan Butterfield, University of Kentucky*  
12:00 - 1:30 Lunch

#### MICRONUTRIENTS AND IMMUNE FUNCTION

*Chair: Emily Ho, Linus Pauling Institute, Oregon State University*

- 1:30 - 2:00 Vitamin D and Immune Function  
*Adrian Gombart, Linus Pauling Institute, Oregon State University*  
2:00 - 2:30 Vitamin A in Immune Function  
*Charles Stephensen, University of California-Davis*  
2:30 - 3:00 Micronutrient Supplementation and Infectious Disease Outcome  
*Wafaie Fauzi, Harvard University*  
3:00 - 3:30 Coffee/Tea Break

#### VITAMIN K: NEW FUNCTIONS AND MECHANISMS OF ACTION

*Chair: Maret G. Traber, Linus Pauling Institute, Oregon State University*

- 3:30 - 4:00 Determinants of Vitamin K Status in Humans  
*Sarah Booth, Jean Mayer USDA Human Nutrition Research Center on Aging, Tufts University*  
4:00 - 4:30 Vitamin K: The Coagulation Vitamin that Became Omnipotent  
*Leon Schurgers, University of Maastricht, The Netherlands*  
4:30 - 5:00 Vitamin K-dependent Protein Carboxylation  
*Kathleen L. Berkner, Case Western Reserve University*  
5:00 - 6:30 Poster Session

### Friday, May 15, 2009

- 6:00 AM Organized Walk/Run  
7:30 Breakfast

#### DIET AND LIFESTYLE IN METABOLIC SYNDROME AND CARDIOVASCULAR DISEASES

*Chairs: Donald Jump, Linus Pauling Institute, Oregon State University and Jeffrey Blumberg, Tufts University*

- 8:30 - 9:00 Diet, Weight Control, and Metabolic Syndrome  
*Jonathan Purnell, Oregon Health & Science University*  
9:00 - 9:30 TBA

9:30 - 10:00 Comprehensive Metabolic Analysis for Understanding of Diabetes and Obesity Mechanisms

*Chris Newgard, Duke University*

10:00 - 10:30 Coffee/Tea Break

10:30 - 11:00 Exercise-induced Protection Against Insulin Resistance

*Deborah M. Muoio, Duke University*

11:00 - 11:30 Dietary and Genetic Effects on Atherogenic Dyslipidemia

*Ron Krauss, Children's Hospital Oakland Research Institute*

11:30 - 12:00 PM The Impact of Obesity on Inflammation and Immune Defense

*Pam Fraker, Michigan State University*

12:00 - 1:30 Lunch

### **EPIGENETICS, DIET, AND DISEASE**

*Chairs: Rod Dashwood, Linus Pauling Institute, Oregon State University and Sharon Ross, National Cancer Institute*

1:30 - 2:00 Epigenetic Programming *In Utero*

*Rebecca Simmons, University of Pennsylvania*

2:00 - 2:30 DNA Methylation, Insulin Resistance, and Blood Pressure in Offspring Determined by Maternal B Vitamin and Methionine Status

*Lorraine E. Young, University of Nottingham, UK*

2:30 - 3:00 Dietary HDAC Inhibitors in Garlic and Broccoli  
*Rod Dashwood, Linus Pauling Institute, Oregon State University*

3:00 - 3:30 Oxidative Stress, Dietary Polyphenols, and Epigenetics in Lung Aging  
*Ifan Rahman, University of Rochester Medical Center*

3:30 - 4:00 Coffee/Tea Break

### **AWARD CEREMONY FOR THE LINUS PAULING INSTITUTE PRIZE FOR HEALTH RESEARCH**

4:00 - 5:30 Presentation by LPI Director, Balz Frei  
Plenary Lecture by Awardee

6:30 Reception

7:00 Banquet

**Saturday, May 16, 2009**

8:00 AM Breakfast

### **PUBLIC SESSION**

*Chair: Balz Frei, Linus Pauling Institute, Oregon State University, Corvallis, OR*

9:00 - 11:00 What to Eat?

*Marion Nestle, New York University*

**For more information about the Conference, please see the LPI Web site at <http://lpi.oregonstate.edu> or phone the Institute at 541-737-5075.**

### *Continued from page 7 — Micronutrients for Older Adults*

reduced intestinal absorption, and increased urinary losses of the mineral. Magnesium deficiency can lead to a number of health problems because the mineral plays several diverse roles in the body. Magnesium is a cofactor for more than 300 metabolic reactions, including those required for energy production, nucleic acid (DNA and RNA) synthesis, and protein synthesis. Moreover, magnesium is necessary for normal muscle and nerve function, as well as bone health. In fact, about 60% of all magnesium in the body is located in the skeleton, where it plays a structural role. Magnesium deficiency can impair calcium and vitamin D metabolism, leading to bone loss. Some studies have found that inadequate dietary intake of magnesium and/or low serum levels of magnesium are associated with reductions in bone mineral density. It is not yet known whether magnesium supplementation could prevent loss of bone integrity. Few multivitamin-mineral supplements contain more than 100 mg of magnesium because the resulting pill would be too bulky. Because older adults are more likely to have impaired kidney function, they should avoid taking more than 350 mg/day of supplemental magnesium without medical consultation. Eating magnesium-rich foods is safe because, in contrast to supplemental magnesium salts, adverse effects have not been identified from magnesium occurring naturally in food.

Together, adequate nutritional status of calcium, vitamin D, and magnesium is necessary for maintenance of bone health and prevention of bone-related diseases in older adults.

### **Micronutrients that function as antioxidants**

Vitamins C and E have several functions in the body and have major roles as antioxidants.

#### ***Vitamin C***

Vitamin C is the most effective water-soluble antioxidant in human plasma. An antioxidant is a substance that prevents or reduces damage caused by free radicals that are generated during normal cellular metabolism and upon exposure to environmental toxins, such as cigarette smoke. Vitamin C and other antioxidants protect various molecules in the body, including proteins, lipids, and nucleic acids (DNA and RNA), from oxidative damage by free radicals. While it is not yet known whether older adults have higher vitamin C requirements, some older populations reportedly have daily intake levels lower than the current recommended dietary allowance (75 mg/day for women and 90 mg/day for men). The RDA, set by the Food and Nutrition Board of the Institute of Medicine, is based on prevention of a deficiency and not on the prevention of chronic diseases or the promotion of optimum health. Studies conducted at the National Institutes of Health indicate that plasma and circulating cells in healthy, young subjects attain maximal

*continued on page 10*

concentrations of vitamin C at a dose of about 400 mg/day—a dose much higher than the current RDA. Pharmacokinetic studies in older adults have not yet been conducted, but evidence suggests that the efficiency of one of the molecular mechanisms for the cellular uptake of vitamin C declines with age. Because maximizing blood levels of vitamin C may be important in protection against oxidative damage to cells and biological molecules, a vitamin C intake of at least 400 mg daily is particularly important for older adults who are at higher risk for chronic diseases caused, in part, by oxidative damage, such as heart disease, stroke, or cataract.

### Vitamin E

Vitamin C is able to regenerate another dietary antioxidant, vitamin E, from its oxidized form. Vitamin E actually refers to a family of eight fat-soluble antioxidants, but alpha-tocopherol is the only form that is actively maintained in the body. Alpha-tocopherol functions as an antioxidant in lipid compartments of the body, quenching the propagation of lipid peroxidation in cellular membranes. In addition, alpha-tocopherol prevents the oxidation of fats in low-density lipoprotein (LDL). LDL transports cholesterol from the liver to the peripheral tissues. Because oxidized LDL has been implicated in the development of atherosclerosis, several studies have examined whether vitamin E is protective against cardiovascular diseases. Moreover, since the brain is particularly vulnerable to oxidative stress, vitamin E may also help prevent neurodegenerative diseases. It has been estimated that more than 90% of Americans do not meet the dietary recommendations for vitamin E—15 mg (22.5 IU) daily for all adults. A daily supplement of 200 IU of natural source d-alpha-tocopherol (RRR-alpha-tocopherol), taken with a fat-containing meal, may help protect adults from chronic diseases.

### B vitamins

Compared to younger adults, older adults may have increased requirements for some of the B vitamins that are involved in energy production and other metabolic reactions.

#### Vitamin B<sub>6</sub>

Vitamin B<sub>6</sub> has several different functions in the body, participating as a cofactor for more than 100 enzymes.

This vitamin is important in neurotransmitter synthesis, red blood cell formation and function, niacin formation from the amino acid tryptophan, steroid hormone function, and nucleic acid synthesis. Vitamin B<sub>6</sub> intake recommendations for adults older than 50 years are higher than those for younger adults, perhaps because the vitamin's metabolism is altered with aging. Mechanistic studies are needed to determine exactly why older adults require higher intakes. Despite higher recommendations for older adults, several surveys have found that over half of individuals over age 60 consume less than the current RDA (1.7 mg/day for men and 1.5 mg/day for women). Thus, it would be prudent for older adults to take a daily multivitamin supplement, which

generally provides at least 2 mg of vitamin B<sub>6</sub>.

#### Folate

Folate and folic acid are two terms often used interchangeably; the former refers to folates found naturally in foods, while the latter refers to the more bioavailable, synthetic form that is used in vitamin supplements and fortified foods. Dietary intake recommendations for folate are not higher in older adults compared to younger people. However, there is some concern that intake recommendations are not being met in some older adults, especially the elderly, presumably

due to low intake of fruits and vegetables. Even marginal deficiencies can elevate blood homocysteine levels, possibly increasing risk for cardiovascular diseases, as well as Alzheimer's disease and other types of dementia.

#### Vitamin B<sub>12</sub>

Along with folate, adequate intake of vitamin B<sub>12</sub> is necessary to avoid increased blood levels of homocysteine. In humans, vitamin B<sub>12</sub> is only required for two biochemical reactions: to make the amino acid methionine from homocysteine (folate is also needed) and to make a compound that is involved in the production of energy and in the synthesis of hemoglobin, the oxygen-carrying pigment in red blood cells.

The prevalence of vitamin B<sub>12</sub> inadequacy increases with age, making older adults more susceptible to deficiency. If the deficiency is not corrected, anemia as well as neurological and gastrointestinal symptoms can result. Deficiency symptoms may take years to manifest because the vitamin can be stored in the body. Older adults are more vulnerable to vitamin B<sub>12</sub> deficiency because they are more likely to be affected by atrophic gastritis, a chronic inflammation of the

Select Micronutrient Requirements for Older Adults (>50yrs)				
Subject	Micronutrient	IOM Recommendation (RDA except where otherwise noted)		LPI Recommendation
		Men	Women	
Bone metabolism	Calcium	1,200 mg/day (AI)	1,200 mg/day (AI)	Same as IOM
	Vitamin D (51-70 years)	10 mcg (400 IU)/day (AI)	10 mcg (400 IU)/day (AI)	2,000 IU/day from supplements; serum level of $\geq 32$ ng/ml (80 nmol/l).
	Vitamin D (>70 years)	15 mcg (600 IU)/day (AI)	15 mcg (600 IU)/day (AI)	
	Magnesium	420 mg/day	320 mg/day	Same as IOM; no supplement providing $\geq 350$ mg/day
Antioxidants	Vitamin C	90 mg/day	75 mg/day	$\geq 400$ mg/day
	Vitamin E	15 mg (22.5 IU)/day	15 mg (22.5 IU)/day	200 IU/day
B vitamins	Vitamin B <sub>6</sub>	1.7 mg/day	1.5 mg/day	Same as IOM
	Folate	400 mcg/day	400 mcg/day	Same as IOM
	Vitamin B <sub>12</sub>	2.4 mcg/day*	2.4 mcg/day*	100-400 mcg/day of crystalline vitamin B <sub>12</sub>

\*Vitamin B<sub>12</sub> intake should be from supplements or fortified foods due to the age-related increase in malabsorption. Abbreviations: IOM=Institute of Medicine; mcg=microgram; mg=milligram; IU=International Unit; RDA=Recommended Dietary Allowance; AI=Adequate Intake

lining of the stomach, which ultimately results in stomach atrophy and decreased stomach acid production. Reductions in stomach acid impair the release of vitamin B<sub>12</sub> from proteins in food. Thus, food-bound vitamin B<sub>12</sub> malabsorption results in individuals with atrophic gastritis. It has been estimated that 10-30% of older adults are affected by this condition. Therefore, some nutritionists recommend that adults older than 50 years take 100 to 400 mcg of supplemental vitamin B<sub>12</sub> daily. Absorption of the synthetic, crystalline form of vitamin B<sub>12</sub> found in dietary supplements and fortified foods is not impaired by atrophic gastritis.

Daily intake requirements for the select micronutrients mentioned above are listed in the table on page 10. For each micronutrient, the Food and Nutrition Board of the Institute of Medicine establishes a recommended dietary allowance (RDA) or adequate intake (AI) level. The Linus Pauling Institute generally supports the recommendations of the IOM, but any discrepancies in dietary recommendations

are listed in the rightmost column of the table. These and other micronutrients can be obtained by eating nutrient-dense, healthful foods. A daily multivitamin-mineral supplement is important for added insurance that micronutrient requirements are met. In general, men and postmenopausal women should not take a multivitamin-mineral containing iron, and older adults should not take iron supplements unless they have been diagnosed with iron deficiency. Additionally, vitamin A toxicity may occur at lower doses in older adults than in younger adults. The Linus Pauling Institute recommends taking a multivitamin supplement that provides no more than 2,500 IU of vitamin A or a supplement that provides 5,000 IU of vitamin A of which at least 50% comes from beta-carotene. High-potency vitamin A supplements should not be used without medical supervision due to the risk of toxicity. More information on micronutrients for older adults can be found in the LPI Micronutrient Information Center at: <http://lpi.oregonstate.edu/infocenter/olderadultnut.html>. **LPI**



## Copper Chelation and Vascular Inflammation

Hao Wei  
LPI Graduate Research Assistant

*Copper is a double-edged sword: small amounts are required for health, but excess copper can generate free radicals and cause inflammation or toxicity. Tetrathiomolybdate (TTM) chelates copper, helping to remove excess amounts from the body. Copper is required for angiogenesis, or blood vessel formation. Tumors depend on angiogenesis for growth, and copper chelation may be an effective treatment strategy. Treating mice with TTM reduced copper levels by over 50% and inhibited inflammatory molecules that contribute to the development of atherosclerosis.*

### Copper, an essential mineral, can be toxic

Copper is one of the most abundant trace elements found in the human body. It is an essential nutrient and intricately involved in catalyzing biochemical reactions and stabilizing proteins. Copper serves as a cofactor in enzymes involved in cellular energy generation (cytochrome C oxidase), free radical detoxification (copper-zinc superoxide dismutase, or SOD), connective tissue production (lysyl oxidase), iron mobilization (ceruloplasmin), and neurotransmission (dopamine β-hydroxylase). However, excessive copper can be toxic, primarily by causing oxidative damage to the body. Copper is involved in both generation of and defense against reactive oxygen species (ROS). As a transition metal, copper can change its redox status by accepting and donating electrons, shifting between the cuprous (Cu<sup>+</sup>) and cupric (Cu<sup>2+</sup>) forms. Therefore, copper can participate in reactions that generate superoxide radicals and hydrogen peroxide, which are major ROS in the body. If the level and distribution of copper are not carefully regulated, its uncontrolled redox activity can lead to the harmful generation of these ROS, compromising cellular functions. Precise regulatory mechanisms must be in place to prevent the accumulation of copper to toxic levels. Free copper, unbound to proteins, rarely exists in cells, and there are

multiple proteins that serve as copper chaperones by binding to copper and facilitating its distribution. Copper can also exert toxic effects by displacing other essential metal ions from their natural ligands (molecules bound to the metal). The replacement of zinc by copper in the human estrogen receptor renders the protein defective, altering its role in hormone-dependent cell signaling. One proposed mechanism for amyotrophic lateral sclerosis (ALS) is that the loss of zinc from Cu,Zn SOD in motor neurons generates defective SOD, which then produces superoxide radicals instead of scavenging them as a functional antioxidant.

The human body contains 50 to 120 mg of copper, most of which is stored in the liver. Copper is readily absorbed from the diet through the small intestine and is usually excreted via bile through the gastrointestinal tract. The average dietary intake of copper by human adults in the U.S. varies from 1.0 to 1.6 mg/day. The main sources are seeds, grains, nuts, and beans (concentrated in the germ and bran), shellfish, and liver. The recommended copper intake for adults is 0.9 mg/day, and an intake of copper exceeding 10 mg/day may lead to symptoms of toxicity, such as weakness and nausea.

Copper is also required for normal embryonic development and the growth of new blood vessels (angiogenesis). Interest in copper's role in angiogenesis dates back several decades. Researchers found that copper promoted angiogenesis in the rabbit cornea and that copper deficiency reduced angiogenesis produced by a known angiogenic stimulant. Because tumors require new blood vessel growth to obtain nutrients and remove waste, anti-angiogenic strategies are important in cancer therapy. Clinical studies have demonstrated the potential of copper chelators for the treatment of cancer in humans.

Recent studies show that copper is also implicated in inflammatory conditions, such as pulmonary fibrosis and arthritis. Using copper chelators, researchers demonstrated that production of many cellular growth factors, interleukins, and pro-inflammatory cytokines, such as tumor necrosis factor-alpha, are affected by copper. Reduction of intracellular copper leads to a decrease of these growth

*continued on page 12*

factors, interleukins, and cytokines. These copper-regulated molecules are involved in multiple physiological functions, such as tissue growth, angiogenesis, and inflammation. Angiogenic and inflammatory signaling pathways are related, and the critical regulator orchestrating both pathways is the transcription factor NF- $\kappa$ B, a protein that can regulate gene expression by binding to a specific DNA sequence. Although the mechanism of copper's role in promoting angiogenesis and mediating inflammatory events is not totally understood, it has been suggested that NF- $\kappa$ B may be the critical factor affected by copper.

## Atherosclerosis is an inflammatory disease

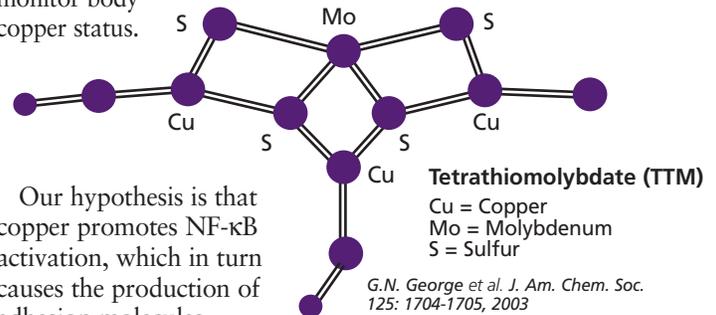
Atherosclerosis is the leading cause of mortality in the Western world and is increasing in developing countries. Atherosclerotic lesions begin to form early in life, develop progressively, and most often remain clinically insignificant until the acute rupture of a plaque and the ensuing thrombosis, leading to myocardial infarction or stroke. Although atherosclerosis was formerly considered as simply a lipid storage disease, we now appreciate the role of inflammation in atherosclerosis. Compelling evidence indicates that inflammation plays a major role in all stages of atherosclerosis—from initiation to thrombotic complications—that are characterized by intense immunological activity. Levels of inflammatory markers also predict the outcome of patients with acute coronary syndrome.

These inflammatory markers include adhesion molecules secreted by cells in the vascular wall, chemokines, and pro-inflammatory cytokines. The earliest development of atherosclerosis is characterized by inflammatory events and the recruitment of blood cells, such as monocytes, into the vascular wall of the major arteries. Adhesion molecules and chemokines secreted by vascular cells cause monocytes to roll along the vascular wall and then migrate across the endothelial layer into the intima, the innermost layer of the vessel wall. There, the monocytes differentiate into macrophages and absorb oxidized lipids. These lipid-laden macrophages, also known as foam cells, are the major component of the fatty streak, which is the hallmark of early atherosclerosis. During these processes, NF- $\kappa$ B is significantly activated and, in turn, increases the levels of other inflammatory molecules to mediate further monocyte recruitment to the vessel wall. The progressive development of lesions is characterized by inflammation, lipid accumulation, cell death, and fibrosis. Over time, these lesions develop into mature atherosclerotic plaque that can cause a heart attack or stroke.

## Copper chelation with tetrathiomolybdate

The term chelate, from the Greek “chele” for “claw”, refers to the ability of a substance to combine with metals in the body so that they can be excreted. Metal chelation therapy is traditionally used in occupational medicine, as it effectively removes toxic heavy metals from the body. Chelation therapy is also used as a complementary or alternative treatment in an attempt to inhibit oxidative stress, atherosclerosis, or tumor growth. Metal chelation is also used to study the biological function of metal ions in living systems.

Tetrathiomolybdate (TTM) is an anti-copper drug initially developed to treat Wilson's disease, a genetic disease characterized by excessive accumulation of copper in the liver and consequent liver and brain damage. The copper-chelating property of TTM was discovered decades ago in Australia and New Zealand, where ruminants developed copper deficiency due to naturally formed TTM in the sulfur-rich rumen. TTM is a small compound with high affinity and specificity to bind copper. It forms a stable complex with copper and protein, and one TTM molecule can chelate up to three copper ions (see figure below). TTM is fast acting and has relatively low toxicity among copper chelators. Its toxicity is mainly due to copper deficiency, which can be easily reversed by short-term copper supplementation. Administered orally, TTM can bind copper in food and prevent it from being absorbed. Recently, TTM has been shown to have anticancer activity in rodents through the inhibition of angiogenesis. As indicated above, tumor angiogenesis requires copper, so the strategy is to lower copper to levels that impair angiogenesis in tumors but still meet copper requirements in normal cells. During TTM treatment, the level of ceruloplasmin, a copper-containing protein in blood, is commonly used as a surrogate marker to monitor body copper status.



Our hypothesis is that copper promotes NF- $\kappa$ B activation, which in turn causes the production of adhesion molecules, chemokines, and pro-inflammatory cytokines during vascular inflammation, and, therefore, copper chelation by TTM ameliorates inflammatory conditions by inhibiting NF- $\kappa$ B activation. We first studied TTM's anti-inflammatory effects on human aortic endothelial cells (HAEC) in culture. By treating HAEC with TTM, we successfully suppressed the inflammatory response induced by exposure to the endotoxin lipopolysaccharide (LPS). We observed inhibition of the production of adhesion molecules, chemokines, and pro-inflammatory cytokines, as well as inhibition of NF- $\kappa$ B activation. We then fed TTM to mice every day for three weeks. Following an acute inflammatory stimulation with LPS, we checked toxicity, inflammatory response, copper, and the activity of inflammation-related transcription factors. TTM-treated mice showed no side effects of any kind, and no liver toxicity was observed. Following TTM treatment, serum copper was reduced by 57%. We found that TTM inhibited the expression of adhesion molecules, chemokines, and pro-inflammatory cytokines, and that NF- $\kappa$ B activity was also significantly decreased by TTM. Our studies support the concepts that copper plays a role in mediating vascular inflammation by promoting NF- $\kappa$ B activation and that TTM can inhibit acute inflammation by decreasing NF- $\kappa$ B activity. TTM chelation of excessive copper associated with pathological development has the potential to treat atherosclerosis and ameliorate other inflammatory conditions in the vascular system. We are currently investigating the anti-atherosclerotic potential of TTM in a mouse model of human atherosclerosis. **LPI**



## Triglyceride-lowering Properties of Lipoic Acid

Régis Moreau, Ph.D.  
LPI Assistant Professor (Sr. Res.)

*Chronically elevated triglyceride levels in the blood are associated with metabolic syndrome, diabetes, and heart disease. Triglycerides come from the diet and are made in the liver. Obesity, diabetes, a high-fat diet, and various genetic conditions can cause elevated triglycerides. When fed to rats capable of becoming hypertriglyceridemic, lipoic acid, a naturally occurring antioxidant, prevented elevated triglyceride levels and increased HDL (the good cholesterol) levels by over 20%. Lipoic acid apparently works by inhibiting the synthesis of triglycerides in the liver.*

The condition of abnormally high blood triglyceride levels (>150 mg/dl) is known as hypertriglyceridemia. Recent estimates indicate that about 30% of U.S. adults have hypertriglyceridemia. Triglycerides (also known as triacylglycerols) are composed of three long-chain fatty acids esterified to glycerol. Animal fats and vegetable oils contain mixtures of triglycerides, which differ by the chain length of their fatty acids and the presence or absence of double bonds in the molecule. Triglyceride is the most concentrated biological fuel—making it an ideal form of energy storage.

There are two main sources of triglycerides: the diet and the liver. Dietary triglycerides are absorbed in the small intestine, assembled into chylomicrons in the intestinal mucosa, and secreted into the lymph. They then enter the blood via the thoracic lymph duct. Muscle and adipose tissues remove some of the triglycerides from the chylomicrons, producing chylomicron remnants that are accumulated in the liver. The liver also produces triglycerides from both fat and carbohydrate. Hepatic triglycerides are then packaged into very low-density lipoproteins (VLDL) and secreted into the blood for delivery to various tissues for the production of energy. Hence, VLDL are the lipoprotein particles formed to transport endogenously derived triglycerides to tissues. Food intake in excess of the body's need for energy exacerbates fat deposition in adipose and muscle tissue, with possible detrimental health consequences.

Hypertriglyceridemia is usually discovered after performing a routine lipid profile. Hypertriglyceridemia stems from disturbances in the synthesis and/or degradation of triglyceride-rich lipoproteins and may be the result of various genetic defects and/or a high-fat diet, obesity, diabetes, hypothyroidism, or certain medications. Hypertriglyceridemia is a key component of metabolic syndrome and type 2 diabetes and is also strongly correlated with increased risk of cardiovascular diseases. In its most severe forms, hypertriglyceridemia (500-3,000 mg/dl) may cause pancreatitis, eruptive xanthomas (yellow papules underneath the skin), or lipemia retinalis (creamy white appearance of the blood vessels of the retina).

Conventional therapy for hypertriglyceridemia includes dietary weight loss and exercise, dietary supplementation with fish oil or niacin, and drug intervention, which may include fibrates or combined therapy with statins when low-density lipoprotein (LDL) is elevated (see Table). Although the National Cholesterol Education Program

Adult Treatment Panel III guidelines recommend the use of fibrates in combination with statins in patients at very high risk of coronary heart disease (for example, hypertriglyceridemic patients at the target level for LDL-cholesterol with low HDL-cholesterol), many physicians remain reluctant to use these drugs due to concerns about muscle dysfunction (myopathy rhabdomyolysis) and the lack of definitive evidence that hyperlipidemia can be controlled with fibrates. As a direct result of these concerns, the Action to Control Cardiovascular Risk in Diabetes (ACCORD) is currently the largest clinical trial ever (10,000 people) to test the efficacy and safety of a fenofibrate/statin combination, with the primary results to be announced in early 2010.

The evidence cited above has led scientists to appreciate the need for complementary therapeutic strategies in the prevention and treatment of hypertriglyceridemia and obesity-induced hyperlipidemia. Recent findings support the novel role of lipoic acid in reducing the metabolic risk factors associated with obesity. Lipoic acid, a naturally occurring antioxidant that serves as a cofactor for mitochondrial enzymes, has long been reported to improve glucose uptake in diabetes and has been prescribed by physicians, especially in Germany. Green leafy vegetables are a good source of lipoic acid. However, the levels needed to exert the metabolic effects discussed here are not commonly achieved dietarily.

Selected therapies for managing hypertriglyceridemia				
Therapy	Triglyceride	LDL-cholesterol	HDL-cholesterol	Possible side effects
Statins	▼20-40%	▼18-55%	▲ 5-15%	Myopathy rhabdomyolysis, elevated liver enzymes
Fibrates	▼40-60%	▲ 5-30%	▲ 15-25%	Rhabdomyolysis
Niacin	▼30-50%	▼ 5-25%	▲ 20-30%	Flushing, worsening glycemia, elevated liver enzymes
Fish Oil	▼30-50%	▲ 5-10%	▲ 5-10%	Fishy aftertaste, gastrointestinal upset

Adapted from Oh et al. *American Family Physician* 75:1365-1371, 2007

Although known for its role in glucose uptake, the triglyceride-lowering properties of lipoic acid were only recognized recently. Our studies at the Linus Pauling Institute have contributed to this growing body of knowledge. We added lipoic acid to food fed to Zucker Diabetic Fatty (ZDF) rats, which are used to study hypertriglyceridemia and type 2 diabetes. Typically, ZDF rats become hypertriglyceridemic due to overproduction of triglyceride-rich lipoproteins in the liver, while triglyceride degradation remains normal. When fed a carbohydrate-rich diet, ZDF rats predictably develop hypertriglyceridemia (up to 800 mg triglyceride/dl) at 10 weeks of age and type 2 diabetes (500 mg glucose/dl) at 12 weeks of age. Since ZDF rats are hypertriglyceridemic before becoming fully diabetic, we fed them lipoic acid (2.4 grams per kg of food) from the age of 5 weeks until the age of 10 weeks, thus eliminating potential confounding effects due to diabetes. That dose roughly equates to a daily dose of 2 grams of lipoic acid in humans.

*continued on page 14*



During the course of our experiment, we observed a rise in blood triglycerides (451 mg/dl at 9 weeks of age) in control rats not fed lipoic acid. However, no such increase was detected in the rats fed lipoic acid. We also observed

that the rats fed lipoic acid consumed less food than the control rats. Because meal size could affect blood lipid levels, a group of rats was fed a restricted amount of the control diet (without lipoic acid) to match the food intake of the rats fed lipoic acid. This treatment, called pair-feeding, ensures that comparisons between groups are based on the same caloric intake. Despite food restriction, pair-fed rats still developed hypertriglyceridemia (398 mg/dl at 9 weeks of age), indicating that the lipid-lowering properties of lipoic acid extend beyond its effect to curb appetite. Hence, subsequent comparisons were made between lipoic acid-fed and pair-fed rats.

In addition to normalizing blood triglycerides, lipoic acid significantly raised high-density lipoprotein (HDL)-cholesterol (the so-called good cholesterol) by 23% with no marked changes in blood total cholesterol. By using the blood triglyceride to HDL-cholesterol ratio as a predictive index of atherogenesis, lipoic acid would decrease the risk for coronary artery disease in ZDF rats by 70%. It is important to remember that these effects of lipoic acid on blood lipids occurred while the ZDF rats were pre-diabetic. Glycemia was not significantly different between experimental groups, nor was glycemia significantly elevated compared to age-matched, lean Zucker rats with normal blood sugar (147 mg glucose/dl).

Next, we identified the triglyceride-rich lipoproteins affected by lipoic acid. Chylomicrons and VLDL are the primary lipoprotein carriers of triglycerides in the blood. Our data showed that lipoic acid decreased circulating VLDL by about 50%, at least in part, by repressing VLDL secretion by the liver. Our data also suggest that lipoic acid lowered blood chylomicrons following a meal. This means that lipoic acid affects triglycerides from both dietary and endogenous origins.

Further analyses of triglyceride synthesis in the liver showed that lipoic acid decreased the gene expression of key enzymes of triglyceride synthesis, namely *sn*-glycerol-3-phosphate acyltransferase-1 by 81% and diacylglycerol O-acyltransferase-2 by 56%. This result was associated with a decrease in liver triglyceride content by 35% and supports the notion that lipoic acid inhibits liver triglyceride synthesis. Since lipoic acid also decreased the rate of triglyceride-rich VLDL secretion from the liver by 31%, the results further suggest that lipoic acid prevents hypertriglyceridemia by interfering with the liver's ability to synthesize and secrete triglycerides. Because triglyceridemia reflects the balance between

triglycerides that are absorbed and synthesized and those that are cleared by peripheral tissues (for example, skeletal muscle, adipose tissue, and heart muscle), we determined whether lipoic acid stimulated the elimination of triglycerides in skeletal muscle. Results obtained thus far suggest that skeletal muscle may be a target tissue for fat elimination in lipoic acid-fed ZDF rats. At the end of the 5-week feeding period, we noted that lipoic acid-treated rats had significantly less perivisceral fat, but their livers were heavier than those of control rats. While a reduction in body fat is certainly seen as positive, the health repercussions of an enlarged liver are uncertain and require further analysis. The anatomical inspection of the livers and measurement of lipid content excluded the possibility of lipoic acid-induced liver steatosis (fatty liver). On the contrary, lipoic acid markedly lowered total lipid concentration by 26% in the liver of ZDF rats, including triglycerides. Moreover, a marker enzyme of liver function (alanine aminotransferase) was normal, indicating that lipoic acid did not cause liver damage. Liver glycogen content was increased by 27% in lipoic acid-fed rats, suggesting that lipoic acid induced the storage of dietary carbohydrates as glycogen instead of being converted into fat. Within certain limits, glycogen deposit is viewed as a healthier alternative to fat deposit. Since glycogen retains significant amounts of water, glycogen and its water content may, at least in part, account for the increase in liver size.

Currently, we are investigating the molecular mechanism of lipoic acid on triglyceride synthesis in rat livers and cultured liver cells. Ongoing studies seek to determine the precise role of known regulatory proteins on key enzymes in the triglyceride synthetic pathway and how lipoic acid may modulate their functions.

In summary, the study showed that the progression of hypertriglyceridemia could be halted by feeding lipoic acid to ZDF rats. Lipoic acid-treated animals were leaner and showed improved blood VLDL- and HDL-cholesterol profiles and a healthier plasma atherogenic index. The inhibition of liver triglyceride synthesis played an important part in the triglyceride-lowering properties of lipoic acid. Since lipoic acid appears to regulate triglyceride metabolism in a manner distinct from prescribed drugs, its mechanism of action should be further investigated. Given its strong safety record in humans, lipoic acid may have promising therapeutic applications against hypertriglyceridemia and hyperlipidemia. **LPI**





# Developments

Scott Palmer  
LPI Director of Development

## Linus Pauling Institute Launches \$1.5 Million Fund-Raising Campaign

With the completion of the Schematic Design Phase this past month, plans for the new Linus Pauling Science Center at OSU made one giant step forward. Next will be the Design Development Phase, which will lead to the drafting of the construction documents and the beginning of the competitive bidding process for contractors by early spring. Groundbreaking is planned for June of 2009, and construction should take just under two years.

Half of the \$62 million needed to build this 102,000 square foot facility will come from the state of Oregon, and the other half has been donated, including a \$20 million grant from the Wayne and Gladys Valley Foundation and a \$10.65 million gift from Al and Pat Reser, two OSU alumni. For us here at the Institute, the Linus Pauling Science Center is more than just a new building – it is the key to achieving a new level of creativity and innovation. Dr. Pauling believed deeply in the value of scientific collaboration and academic collegiality. When this incredible new facility is completed in 2011, we will finally realize his vision by bringing our scientists together under one roof.

Major decisions regarding space allocation were made during the Schematic Design Phase. We now have a much better idea of the layout within the Linus Pauling Science Center and how functional space will be organized. This part of the design process allowed us to identify unique opportunities that, if included now, will significantly enhance the long-range, positive impact the building will have on our research.

Because of the way the funding package for the Linus Pauling Science Center was developed, the additional costs for these unique opportunities cannot be included within the \$62 million project cost. Yet these enhancements are so critical to our future research that we have launched a building enhancement campaign to raise \$1.5 million in gifts from our friends and donors to ensure they are included in the building.

Funds from the Linus Pauling Science Center Enhancement Campaign will be used to buy new high-tech research equipment for the three core laboratories in the new building, to purchase mobile lab benches that provide maximal flexibility in configuring labs and enhance collaborative research, and to build state-of-the-art animal facilities that provide the best care for the animals and comply with the highest standards of the Association for Assessment and Accreditation of Laboratory Animal Care.

All three major research areas within the Institute—the Cancer Chemoprotection Program, Cardiovascular and Metabolic Diseases, and the Healthy Aging Program—will benefit significantly from these additions.

All gifts of \$10,000 or more will be recognized on a Donor Wall that will be located on the main floor of the building. For gifts of \$25,000 and up, opportunities to name rooms within the Linus Pauling Science Center are available. There are a wide variety and range of naming opportunities, including offices (\$25,000 to \$50,000), meeting rooms (\$100,000 to \$500,000), research labs (\$250,000), or an entire floor (\$1,000,000)!

You can participate in this special campaign in a number of different ways — an outright gift of cash or a pledge that is payable over several years, gifts of stocks or real estate, or even a disbursement from your IRA. On October 3<sup>rd</sup>, Congress and the President approved legislation that includes retroactive extension of the popular IRA Charitable Rollover of 2006. This means that in 2008 and 2009 donors who are 70½ or older can transfer up to \$100,000 per year per person from their IRA directly to a charity without paying income tax on the money.

Gifts can only be made from an IRA. Pension, 401k, profit sharing, and other forms of retirement funds do not fall under this legislation. However, donors can roll non-qualifying accounts (for example, 401k) into the qualifying IRA account in order to make a gift. You can give to multiple charities in any combination that does not exceed the \$100,000 per year per person limit, and you can use your donation to fulfill your yearly required minimum distribution in part or in full.

Gifts can only be made directly to public charities to be eligible for tax benefits. A gift cannot be made to a charitable remainder trust or charitable gift annuity. Of course, you can use your IRA gift to name a room in the new Linus Pauling Science Center.

We would be happy to help you make an IRA charitable rollover gift by providing you with a letter of instruction for you to fill out and send to your IRA custodian. If you would like more information on how you can help ensure the future of the Linus Pauling Institute through an IRA gift; a gift of real estate, stocks or mutual funds; and perhaps name a room in the Linus Pauling Science Center, please contact me toll free at (866) 218-8930, or on my direct line (541) 231-6751.

With your help, the Linus Pauling Science Center will realize the dreams and vision of our faculty and provide a working memorial to our founder, Linus Pauling. **LPI**

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

Wayne Haag	Fred Kister
Arnold Molzon	Sodya-Sonia Renic



# 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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Tentative design for  
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Page 1 ..... *From the Director*

Page 1 ..... *Zinc: From Diabetes to Cancer—  
An Interview with Emily Ho, Ph.D.*

Page 2 .... *Dr. Adrian “Fritz” Gombart  
Joins LPI*

Page 6 .... *Comparisons of Serum  
Micronutrients between Okinawan  
and Oregonian Elderly*

Page 7 .... *Micronutrients for Older Adults*

Page 8 ..... *Diet and Optimum Health  
Conference*

Page 11 ... *Copper Chelation and  
Vascular Inflammation*

Page 13 ... *Triglyceride-lowering Properties  
of Lipoic Acid*

Page 15 ... *Developments*

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# Linus Pauling Institute