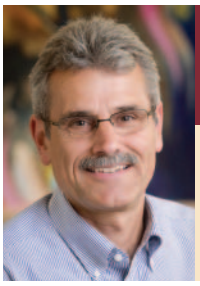
FALL/WINTER 2012

OSU Oregon State University

The Linus Pauling Institute

RESEARCH NEWSLETTER



From the Director

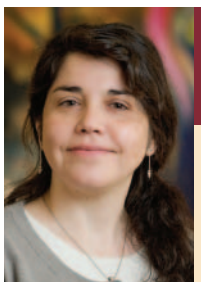
Balz Frei, Ph.D.
LPI Director and Endowed Chair
OSU Distinguished Professor of Biochemistry and Biophysics

It's been a year since we moved into our new home, the Linus Pauling Science Center at Oregon State University. We are very pleased to work in such modern, state-of-the-art facilities, which have transformed the Institute and elevated it to new heights. The open laboratories and shared core facilities; the bright, well-designed offices and conference rooms; and the informal meeting spaces throughout the building have created a stimulating intellectual environment and facilitated new interactions and collaborations. This has resulted in new collaborative research projects, joint publications, and multi-investigator grant applications.

It feels like we have finally arrived—we have achieved the vision we had when the Institute moved here to OSU from Palo Alto many years ago. This vision was to have a state-of-the-art research and teaching facility where top-notch faculty and their students and post-docs, supported by an efficient administrative team, work together to discover new ways of solving some of the most pressing health-care problems facing our nation. We all share a conviction that prevention is the medicine of the 21st century, and that nutrition, micronutrients, and lifestyle are key to improving public health and getting the spiraling health-care costs under control.

The overarching goal of our work in the Institute is to help people extend their healthy lifespan (healthspan)—how we can stay healthy and vital up to an old age. Health in this context is not just defined as the absence of chronic disease but also the absence of infirmity and deficits of daily living as we get older, as well as "a state of complete physical, mental, and social well-being," as the World Health Organization defines it. For example, research in our Healthy Aging Program is discovering new "age-

continued on page 2



Healthy Aging

An interview with Viviana Perez, Ph.D.
Assistant Professor of Biochemistry and Biophysics
LPI Principal Investigator

Q. You're from South America. Were you born and raised in Chile?

A. Yes. I was born in Santiago, and I lived there until I moved to San Antonio, Texas, in 2004.

Q. Where did you attend college?

A. I went to the University of Chile and also did my Ph.D. there.

Q. In what subject did you earn your Ph.D.?

A. Biomedical science. At that time I was working with Dr. Sierra investigating the function of one protein that is overexpressed with age in rats.

Q. What brought you to the United States?

A. Aging research.

Q. Where did you go for that?

A. I came to the University of Texas Health Science Center at San Antonio.

Q. What attracted you to the Linus Pauling Institute?

A. The diversity, the emphasis on healthy aging and micronutrients, and the future application to translate my research to humans.

Q. And you didn't feel you could do that as successfully at your institution in Texas?

A. The strength of the Barshop Institute for Longevity and Aging Studies at the University of Texas, where I worked, is the study of the basic biology of aging, not on translational research.

Q. Do you continue to collaborate with your colleagues there?

A. Yes, I do. I am constantly talking with them, and we are publishing papers together.

continued on page 3

Continued from cover — From the Director

essential micronutrients"—compounds like carnitine, lipoic acid, and coenzyme Q that we synthesize in our body and obtain from our diet in sufficient amounts when we are young but have a hard time synthesizing and absorbing as we get older. To overcome these limitations and assure optimum functioning of all cells and tissues, we may have to take supplements of these compounds.

Age also may increase the need for certain vitamins and nutritionally essential minerals. For example, older adults often do not get enough vitamin C from their diet and may have lower intestinal absorption of vitamin C than younger people. The capacity of our skin to synthesize vitamin D upon sun exposure also declines with age. Since both vitamins C and D are important for good immune function, inadequate levels in our bodies may lead to increased susceptibility to infectious diseases in older adults. An efficient immune system also is important for surveillance and elimination of malignant cells, keeping cancer at bay.

Another interesting connection exists between vitamin C and carnitine in that vitamin C is required for two enzymes in the biosynthetic pathway of carnitine. Carnitine is required for the transport of fat into mitochondria where it is oxidized ("burned"), releasing energy required for normal metabolism and good functioning of our body. Hence, inadequate intake of vitamin C may lead to lack of energy in older adults, and vitamin C or carnitine supplementation may help overcome this deficit.

Two recent studies related to the topic of healthspan caught my eye. The first one, conducted at the Cooper Clinic in Dallas, Texas, investigated the relationship between "cardiorespiratory fitness" in middle-aged adults and the development of chronic diseases later in life. Treadmill time was measured in over 18,000 healthy men and women when they were at a median age of 49. After a median time of 29 years, the prevalence of eight chronic conditions in these individuals was assessed: congestive heart failure, ischemic heart disease, stroke, type II diabetes, chronic obstructive pulmonary disease, chronic kidney disease, Alzheimer's disease, and cancer of the colon or lung, the two most common causes of cancer deaths in the U.S.

Not surprisingly, the investigators found that individuals with higher blood pressure, serum total cholesterol, body mass index, serum glucose, and smoking prevalence had a higher risk of developing these chronic conditions. However, individuals with the highest level of midlife fitness had the lowest incidence of chronic conditions and spent the last five years of their lives in much better health than people with the lowest level of fitness at midlife. The authors concluded that "higher midlife fitness may be associated with the compression of morbidity in older age"—in other words, while lifespan was not extended, healthspan was.

The second study was conducted at the National Institute on Aging (NIA) and investigated the effect of caloric restriction on health and survival in rhesus monkeys. Restricting caloric intake by 10-40% has been reported previously to extend lifespan in worms, flies, rats, and mice. But whether the same approach also can extend lifespan in primates, including humans, is not clear. In this study at NIA, monkeys of different ages were fed a diet restricted by 30% in calories and compared to a non-restricted control group. Interestingly, the investigators found that caloric restriction did not extend lifespan. This result is different from that of another study conducted at the Wisconsin National Primate Research Center (WNPRC) that found a significant extension of lifespan in calorically restricted rhesus monkeys. Nevertheless, the NIA study, like the WNPRC study, found a lower incidence of cancer and diabetes in those animals that were calorically restricted. Measures of "metabolic health and overall function" were also improved, including lower body weight; lower serum triglyceride, total cholesterol, and glucose levels; and less oxidative stress.

In trying to explain the difference in results regarding lifespan extension between the NIA and WNPRC studies, the NIA scientists noted that the diets fed to the two groups of monkeys differed significantly. The WNPRC monkeys received a diet containing 28% sucrose, compared to only 4% in the diet given to the monkeys at NIA. The NIA diet, which has a natural ingredient base, also contains fish oil and phytochemicals, including flavonoids, and protein derived from wheat, corn, soybean, fish, and alfalfa. In contrast, the WNPRC diet is a "purified" diet with no added fish oil or phytochemicals, and protein from a single source called lactalbumin. Hence, the authors suggested that the WNPRC monkeys on caloric restriction lived longer than their non-restricted counterparts because they got less of a bad diet that caused the ad libitum-fed (without restraint) control monkeys to die prematurely. In contrast, the NIA monkeys got a healthier diet and lived longer, and under these circumstances, caloric restriction did not extend lifespan. Dr. Viviana Perez further discusses caloric restriction in her interview on page four of this newsletter.

Bottom line: maintaining a healthy body weight is important, but you don't have to starve yourself to get there. A much better way is to be physically active and maintain a high level of fitness throughout life. And diet composition matters for good health. None of this will help you live longer (except for the morbidly obese), but chances are you will live a much healthier and more enjoyable, active life up to an old age. LPI

Continued from cover — Interview with Viviana Perez

Q. How do you like LPI's new building?

A. It's beautiful. I like the big windows, and the laboratories are open, which allows you to interact with people in other laboratories. It's a very nice building.

Q. Functionally, do the laboratories have everything you need to carry out your research?

A. Oh yes, they have everything that we need to do our work.

Q. What got you interested in aging research?

A. When I was in my second year of graduate school, I didn't know what I would like to do. And it was at that time Dr. Sierra moved to Chile. In a seminar, he talked about aging and why aging is important to study. I realized that he was right—we care much about diseases, for example, heart problems and Alzheimer's disease, but those diseases are all associated with aging. If we can delay aging, we can delay not only those diseases but almost all chronic diseases.

Q. So an important risk factor for some of those diseases is advanced age?

A. Exactly.

Q. In your longevity research, you study the naked mole rat. Why is that rodent especially interesting in research on aging?

A. The naked mole rat is interesting because it has a lifespan much longer than expected, considering its body mass. We expect species that have a similar body mass to have similar lifespans, but this is not true for naked mole rats. For example, a mouse with a body mass similar to the naked mole rats lives, in the best conditions, around three and a half years. The naked mole rat lives 30 years! We don't know the exact mechanisms for that yet, but the oxidative stress hypothesis of aging doesn't seem to be applicable to this animal. Interestingly, they live healthily their whole life until the end, when their physiological condition declines rapidly, and then they die. That is what we want to achieve in humans—to maximize "healthspan."

Q. You are a member of the LPI Healthy Aging Program, which focuses on increasing healthspan. What exactly is healthspan?

A. Healthspan is the period of your lifespan when you are productive and without chronic diseases and conditions that limit your daily living. That is what the Healthy Aging Program in LPI wants to achieve—an increase in healthspan. In other words, we would like to compress morbidity so most of your life can be disease-free and full of productive vitality.

Q. If you compress morbidity, it would then occur towards the end of your lifespan instead of being chronic.

A. Exactly! The idea is to delay all chronic diseases until the end and then die. The goal is to add life to your years, not necessarily years to your life.

Q. Are there any other rodents that live nearly as long as the naked mole rat?

A. For rodents, it is the exception. But among other small mammals, like bats, we also observe differences in lifespan—some bats live shorter and some live longer. For example, evening bats live six years, but short tail bats live around 30 years.

Q. Where is the naked mole rat found, and how does it live in its natural environment?

A. It's found in the northeast of south Africa, around Kenya. They live underground where the temperature is around 29-30 degrees Celsius (84-85 degrees Fahrenheit), with 100% humidity.

Q. Since it lives underground, does its environment have elevated carbon dioxide levels?

A. Yes, since they are strictly subterranean, but they seem to be insensitive to CO_2 levels. Dr. Buffenstein from San Antonio has data indicating that these animals may have adapted to their environment by overexpressing Nrf2, a transcription factor that regulates the response to stress.

Q. What do they eat?

A. In the wild, they eat tubers and bulbs. They obtain all the water that they need through their food.

Q. Do they get enough calcium for skeletal development from these tubers? They presumably don't need vitamin D.

A. Yes, their vitamin D levels are very low because they are subterranean. However, when their calcium status was examined, they looked okay. They are not deficient in calcium, and calcium metabolism appears to be independent of vitamin D in these creatures.

Q. You found that the proteasome may play a role in the naked mole rat's longevity. What is the proteasome and how does it affect aging?

A. The proteasome is a mechanism or pathway in cells that degrades abnormal proteins. For example, if you have a damaged protein that got misfolded or aggregated as a result of an oxidative modification, and it cannot be repaired, the ubiquitin proteasome system will get rid of it. If the protein is not in good shape, it will be

continued on page 4

Continued from page 3 — Interview with Viviana Perez

labeled with ubiquitin, which then will be recognized by the proteasome, which degrades it, releasing the amino acids that then can be used to make new proteins.

Q. How does the proteasome work in the naked mole rat?

A. We found that proteasome activity is 30% higher in the naked mole rat compared to mice. We also measured the ubiquitin protein level, which is an indirect measure of proteasome activity. Incredibly, the level of ubiquitinated proteins in the naked mole rat didn't change with age and remained very low, unlike in mice, which is low when young but increases dramatically with age.

Q. How relevant is that to humans?

A. In studies with human skin fibroblasts, researchers found that proteasome activity declines with age. Several publications have shown that proteasome activity in different species declines with age, so a decline in proteasome activity is associated with the aging process in general.

Q. Why would protein stability be beneficial?

A. Proteins actually carry out all the functions in cells. For example, most enzymes are proteins, and if they are not working well, many physiological functions will decline or fail. If the protein gets damaged a little but is still working, the cell is not going to spend energy to repair this damage because it's working adequately. But if the protein is not functional or starts aggregating, it's going to be degraded because it will cause more damage to the overall cellular function.

Q. What damages proteins?

A. Oxidative stress is one of the major causes of damage to proteins.

Q. Is there any method for improving proteasome activity?

A. Dietary restriction increases proteasome activity. Other strategies have been tried in animals, tissues, or cells that increase proteasome activity, but you don't know if they will be safe for humans. There is another mechanism called autophagy that also degrades proteins. It's more versatile than the proteasome. Autophagy not only takes care of damaged proteins but damaged cell organelles as well. This seems more important because, for example, when the mitochondria get old, we need to replace them, and autophagy will accomplish this. It may be better to find ways to improve autophagy rather than proteasome activity in humans.

Q. You've done several studies on thioredoxin, a sulfur-containing antioxidant enzyme, in mice. Does thioredoxin affect lifespan in mice?

A. Yes. We used a transgenic mouse model that has increased levels of thioredoxin. We found that thioredoxin was one of the few enzymes that increased median lifespan in mice. We didn't find a significant increase in maximum lifespan, but the median lifespan was extended, meaning that morbidity was compressed and the healthy part of the aging curve was extended.

Q. The free radical or oxidative stress hypothesis of aging postulates that free radicals causing oxidative stress damage biomolecules like DNA and proteins, and this damage accumulates with time, causing age-related disease and leading to death. How well do you think this hypothesis holds up in light of all the accumulated evidence?

A. During my post-doctoral studies in San Antonio, Texas, I spent most of my time trying to test the oxidative stress theory of aging. From these studies we can say that oxidative stress plays a role mostly in age-related diseases rather than in longevity itself. This was a very important observation. Therefore, based on these and other results, it is necessary to modify this hypothesis. Why? If oxidative stress is really the key for aging, then if you increase antioxidant enzymes that are going to get rid of oxidants like hydrogen peroxide and superoxide, then that should translate into a longer lifespan, but it doesn't. Even though there is less damage to macromolecules like DNA, proteins, and lipids, animals don't live longer if we increase the antioxidant status. So that is telling us that for lifespan itself, oxidative stress is not the problem. But feeding mice a high-fat, unhealthy diet will increase the rate of metabolic disease, and mice deficient in antioxidant enzymes will develop disease earlier than wild-type mice. Transgenic mice that have elevated levels of antioxidant enzymes will be more protected. So antioxidants can help protect against some of the age-related diseases, even if they don't increase lifespan.

Q. You are also interested in the possible link between caloric restriction and lifespan. What is caloric restriction?

A. Caloric restriction is a paradigm that was proposed a long time ago, around 1930 or so. It aims to decrease the amount of calories that an animal consumes, but it is not malnutrition—there are sufficient vitamins and minerals, so it is only the amount of calories that is decreased. This has been well studied in mice and rats and is so far the only paradigm that will increase lifespan and healthspan in several species.

Q. Why would caloric restriction affect lifespan?

A. Nobody knows the specific mechanism, but we know that dietary restriction is an intervention that affects several parameters of aging. For example, dietary restriction decreases oxidative stress and oxidative damage, improves mitochondrial function, increases proteasome activity and autophagy, and improves protein homeostasis. Dietary restriction also improves several physiological parameters, such as insulin sensitivity. In other words, the whole system works better.

Q. In a controlled environment, it is very easy to control the diet and limit the amount of calories that the animals get. Would it be practical for people to adopt this kind of strategy in anticipation of increased lifespan and/or protection against age-related disease?

A. No, I think it would be impractical for most people, mainly because it's not enjoyable. Also, as you mentioned, it is an intervention that has been done in a clean environment that minimizes exposure to pathogens. This is important because caloric or dietary restriction can impair some responses of the immune system. For example, dietary restriction protects against some infections but increases the risk for others. Furthermore, to get the maximum beneficial effect, you have to drastically limit calories, which is not acceptable to most people.

Q. Is that why scientists have looked for chemical compounds that might mimic calorie restriction by engaging similar molecular mechanisms?

A. Yes, that was the goal several years ago when people started looking for mimetics of dietary restriction. Studies in invertebrates that can be easily manipulated genetically have established molecular pathways that are altered by dietary restriction. Certain chemicals or supplements, including resveratrol, metformin, and rapamycin, are being cataloged as dietary restriction mimetics. Of these three, the only one that increased lifespan and healthspan in mice was rapamycin.

Q. What is rapamycin?

A. Rapamycin is an antibiotic found in the soil of Easter Island. It's a natural compound used clinically for organ transplants and for cancer patients because it has immunosuppressive and antiproliferative effects.

Q. What effect does rapamycin have on mice? Are there any side effects?

A. Rapamycin extends lifespan not only in mice but also in invertebrates, such as fruit flies. And in mice, it not only increases maximum lifespan but also increases healthspan. The side effects in mice are cataracts and testicular atrophy.

Q. Does it affect body weight, insulin sensitivity, or antioxidant status?

A. Studies in mice, rats, and humans have shown that rapamycin induces glucose intolerance and insulin resistance. No effects on antioxidant status have been published, and some studies suggest that long-term rapamycin use reduces body weight.

Q. Have people been given rapamycin long enough to observe any adverse side effects like cataracts observed in rodents?

A. Not that I'm aware of. In humans, rapamycin is only given to patients with cancer or used in combination with other drugs for tissue transplantation, and it may be that the treatment is not long enough to observe those side effects.

Q. Why is rapamycin used in those particular medical conditions?

A. For its immunosuppressive and antiproliferative effects. For example, in organ transplant patients, rapamycin is used in combination with cyclosporine. Rapamycin decreases rejection of the new organ, but the mechanism is not really clear. In cancer, it also reduces the proliferation of cancer cells.

Q. What about effects on gene expression? Have they been studied in mice?

A. We have done one study on gene expression in mice, and our results showed that rapamycin has some similarities to dietary restriction. However, dietary restriction has a massive effect on gene expression compared to rapamycin.

Q. You also mentioned resveratrol and metformin. Resveratrol has attracted a lot of attention because it's present in wine and available as a supplement. How effective is resveratrol on some of these parameters compared to rapamycin?

A. Resveratrol mainly works when the animal is under metabolic stress, so it only extends lifespan and healthspan when the animal is fed a high-fat diet. Resveratrol enhances lipid metabolism and other mitochondrial functions in normal mice, but published data show that it doesn't affect lifespan.

Q. There has been some concern about dosage. For instance, when you try to extrapolate to humans, the resveratrol dose that might be needed to achieve results that you observe in rodents would be impossibly high. Is that true?

A. Yes, this is true. The Intervention Testing Program from the National Institute on Aging (NIA) has been testing the effect of resveratrol on lifespan in mice, and so far, they didn't find an effect on lifespan even with high doses.

Q. What do you think about the recently published study by scientists at the NIA that reported that long-term caloric restriction for about 25 years in monkeys did not affect lifespan?

A. It doesn't surprise me! Dietary restriction works in organisms ranging from yeast to mice. The question is whether it will work in our closest biological relatives, the primates. A previous study done in monkeys in Wisconsin had published results different from those we observed in this study from NIA. Why? Well, I think there are several possible reasons. For example, the diet used in the Wisconsin study was high in sucrose, which can be considered a metabolic stress. Also, the genetic background of the monkeys used in the NIA study is diverse compared to the homogenous genetic background of monkeys used in the Wisconsin study, and we know from previous studies done in mice that the benefits of dietary restriction depend on the genetic background of the animal. For example, it was observed that dietary restriction had a deleterious effect in some animals. The "ad libitum" control monkeys in the NIA study were not really fed without restraint; there were some restrictions. So there are differences, including diet, genetic

continued on page 6

Continued from page 5 — Interview with Viviana Perez

background, and other factors, between these two studies—and, therefore, they produced different results.

Q. Where is your research heading?

A. I am very involved trying to establish whether rapamycin is a dietary restriction mimetic. If it is, it has the potential for translation to humans because it is already used clinically. If we can identify molecular mechanisms and ways to diminish side effects, it would be a good option for increasing healthspan in humans.

Q. Obesity is associated with increased oxidative stress and inflammation, both of which are hallmarks of chronic age-related diseases like heart disease, cancer, and diabetes. Does the current obesity problem in the United States concern you with respect to healthspan?

A. I agree that obesity is a big concern for our society and as a researcher, I think we can do something to improve healthspan in this group of people. However, I think it's important to create public consciousness about this problem. For example, we have to educate society about the importance of eating healthful food and exercising.

Q. What do you like to do when you are not in the lab?

A. I take care of my daughter. She keeps me busy! My family likes outdoor activities, and we often ride our bikes. LPI

Briefly. . .

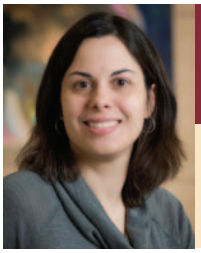
Giana Angelo, Ph.D.
LPI Research Associate
Micronutrient Information Center

Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are very long-chain, highly unsaturated fatty acids derived from alpha-linolenic acid (ALA). ALA is an essential fatty acid and the parent compound from which all omega-3 polyunsaturated fatty acids (PUFAs) are made. Rich dietary sources of ALA are canola oil, flaxseeds, and walnuts. Though EPA and DHA are generated in the body from dietary ALA, they can also be obtained directly from food (especially oily fish) and supplements. The advantage of increasing EPA and DHA content in cells and tissues to improve health and prevent disease is an area of intense research. In particular, the $C_{20\text{-}22}$ omega-3 fatty acids EPA and DHA, often referred to as "fish oils," benefit cardiovascular health by modulating blood lipid levels, inflammation, and the function of endothelial cells (cells that line blood vessels) and cardiomyocytes (heart muscle cells).

A recent systematic review and meta-analysis, published in the *Journal of the American Medical Association (JAMA),* concluded that supplementation with omega-3 PUFAs is not associated with a statistically significant reduction in the risk of major cardiovascular events. A meta-analysis is a statistical method that combines the results from several randomized, clinical intervention trials (RCTs) that address similar questions. Combining multiple trials increases the probability of detecting smaller group differences if present. However, selection criteria for including or excluding studies can strongly influence the results of the meta-analysis and represent an important methodological limitation. In the present analysis, the authors pooled data on major cardiovascular events (all-cause mortality, cardiac death, sudden death, myocardial infarction, and stroke) from 20 RCTs of omega-3 PUFA administration with a combined total of 68,680 patients.

An emerging theme from the *JAMA* study, as well as several other reviews on this topic, is that omega-3 supplementation appears to be better at prevention ("primary prevention") than therapy ("secondary prevention") of cardiovascular diseases (CVD). Notably, the vast majority of the pooled RCTs were secondary prevention trials, meaning that the recruited subjects had pre-existing CVD or were at increased cardiovascular risk. Thus, supplementation with omega-3 PUFAs may not reduce the risk of cardiovascular events in patients with a history of CVD, particularly when used in combination with drug therapy, such as statins, aspirin, or anti-hypertensive medications. However, observational epidemiologic studies have consistently found that increased fish consumption or higher omega-3 PUFA blood levels are associated with a significantly lower risk of cardiovascular events in healthy adults.

Beyond cardiovascular health, EPA and DHA are important for visual and neurological development, exert anti-inflammatory effects, and may slow cognitive decline with aging (see lpi.oregonstate.edu/infocenter/othernuts/omega3fa). If you do not regularly consume fish, LPI recommends a two-gram fish oil supplement several times per week (see lpi.oregonstate.edu/lpirx2). Consumption of fish or fish oil may not be suitable for all individuals, such as vegetarians, vegans, or those with seafood allergies. Alternative sources of $C_{20\text{-}22}$ omega-3 fatty acids, produced in yeast or algae, are commercially available. LPI



Coenzyme Q_{10}

Victoria J. Drake, Ph.D.
Manager
Micronutrient Information Center

Coenzyme Q_{10} is a member of the ubiquinone family of compounds. Humans can endogenously synthesize ubiquinones; therefore, coenzyme Q_{10} is not an essential nutrient. Coenzyme Q_{10} is a fat-soluble compound that is located in cell membranes and lipoproteins. The chemical structure of coenzyme Q_{10} allows it to accept and donate electrons. Coenzyme Q_{10} is part of the mitochondrial electron transport chain, which is a group of electron carriers in mitochondria that transport electrons to and from each other in a sequence in order to generate adenosine triphosphate (ATP)—the energy currency of cells. Coenzyme Q_{10} is also important to help maintain an acid pH in lysosomes, allowing for normal function of enzymes within that organelle that digest cellular debris and waste. Along with vitamin E, coenzyme Q_{10} is an effective antioxidant in cell membranes and lipoproteins.

Although coenzyme Q_{10} is synthesized by the body, it can also be obtained from the diet, with the richest sources being meat, poultry, fish, soybean and canola oils, and nuts. However, the contribution of dietary coenzyme Q_{10} to tissue levels is not clear. Deficiency symptoms have not been reported among healthy individuals, and primary coenzyme Q_{10} deficiency is a rare, autosomal recessive genetic disorder caused by defects in the compound's biosynthetic pathway. This condition is characterized by low tissue levels of coenzyme Q_{10} and, consequently, compromised neuronal and muscular function. Clinical symptoms in some patients with primary coenzyme Q_{10} deficiency improve upon oral supplementation with coenzyme Q_{10}.

Some studies have shown that tissue levels of coenzyme Q_{10} decline with age. Decreased plasma levels of coenzyme Q_{10} have also been observed in patients with diabetes, congestive heart failure, and some cancers. Thus, there is considerable interest in whether oral supplementation with coenzyme Q_{10} can help prevent or treat these and other conditions. Oral supplements have been shown to increase coenzyme Q_{10} levels in plasma and lipoproteins, but it is not yet clear whether oral supplementation in humans increases coenzyme Q_{10} concentrations in other tissues of individuals with normal endogenous coenzyme Q_{10} biosynthesis.

There is little evidence on the effect of coenzyme Q_{10} supplementation on slowing the aging process or preventing chronic diseases. In rodents, lifelong dietary supplementation with coenzyme Q_{10} increased tissue concentrations of the compound and decreased age-related DNA damage; however, lifespan was not extended. To date, research has mainly focused on whether oral coenzyme Q_{10} supplementation could treat various conditions, including genetic mitochondrial disorders, cardiovascular diseases, and neurological diseases.

Mitochondrial encephalomyopathies result from genetic defects in the mitochondrial electron transport chain. Symptoms are improved in some patients with mitochondrial encephalomyopathies after coenzyme Q_{10} supplementation, although patients with genetic defects in coenzyme Q_{10} biosynthesis experience the most dramatic improvement. A phase III clinical trial is presently under way to investigate whether coenzyme Q_{10} might be therapeutic in patients with other mitochondrial disorders.

Because coenzyme Q_{10} levels are decreased in the heart muscle of patients with severe heart failure, several small intervention trials have examined whether coenzyme Q_{10} supplementation improves cardiac function in such patients. A 2006 meta-analysis of ten randomized, controlled trials in heart failure patients found that coenzyme Q_{10} supplementation (99-200 mg/day for one to six months) resulted in a significant, 3.7% improvement in left ventricular ejection fraction, a measure of the heart's pumping ability. Overall, studies to date are mixed, and large-scale, clinical trials are needed to determine whether coenzyme Q_{10} supplementation has utility as an adjunct to conventional therapy in the treatment of congestive heart failure. One such large trial is currently being conducted.

Other studies have looked at whether coenzyme Q_{10} supplementation prevents the free radical-induced heart damage that occurs following a heart attack or during some types of heart surgery, such as coronary artery bypass graft (CABG) surgery. Small, placebo-controlled trials have found that pretreatment with coenzyme Q_{10} (100-300 mg/day for 7-14 days before surgery) improves various short-term outcomes following surgery, such as left ventricular ejection fraction, cardiac output, and postoperative recovery. However, such trials have included relatively few people and have been limited to CABG surgical patients. Studies have also investigated whether coenzyme Q_{10} supplementation (60-600 mg/day) in addition to conventional therapy might help patients with angina pectoris, a condition characterized by chest pain, especially during exercise, due to inadequate oxygen supply to the heart muscle. Most trials have reported improvements in exercise tolerance and electrocardiograms (recordings of the electrical activity of the heart, used to diagnose cardiac arrhythmias, myocardial ischemia, and heart attack) compared to a placebo. Yet, only two studies to date have found coenzyme Q_{10} supplementation to reduce symptom frequency and nitroglycerin consumption.

Hypertension (systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg) affects about one-third of Americans, and nearly as many have pre-hypertension, defined as a systolic blood pressure of 120-139 mm Hg or a diastolic blood pressure of 80-89 mm Hg. A 2007 meta-analysis of 12 clinical trials in hypertensive patients found that coenzyme Q_{10} reduced systolic and diastolic blood pressure by 11-17 mm Hg and 8-10 mm

continued on page 10

7TH INTERNATIONAL CONFERENCE ON

DIET AND OPTIMUM HEALTH

May 15-18, 2013 • Linus Pauling Institute, Oregon State University, Corvallis, OR • USA

Wednesday, May 15

2:00 pm Registration begins at the CH2M Hill Alumni Center on the Oregon State University campus

3:00 **Welcome and opening remarks**
Balz Frei, Conference Chair, Linus Pauling Institute, Oregon State University

OMEGA-3 FATTY ACIDS AND FATTY ACID OXIDATION IN CARDIOVASCULAR AND BRAIN HEALTH

Chairs: Maret Traber and Donald B. Jump
Linus Pauling Institute, Oregon State University

3:15-3:45 **Health benefits of omega-3 fatty acids**
Richard Deckelbaum, Columbia University

3:45-4:15 **Omega-3 fatty acids in the modulation of the inflammatory response and neuroprotection**
Nicolas G. Bazan, Louisiana State University Health Sciences Center

4:15-4:45 **Stearidonic acid, omega-3 index and cardiovascular diseases**
William S. Harris, Health Diagnostic Laboratory, Inc.

4:45-5:15 **Omega-3 fatty acids, cardiac mitochondrial function and heart failure: From lipotoxicity to lipoprotection**
William C. Stanley, University of Maryland

6:30 **Welcome Reception**

Thursday, May 16

6:00 am Organized Walk/Run
7:30-8:30 Breakfast provided

VITAMIN D – HEALTH BENEFITS BEYOND BONE

Chair: Adrian "Fritz" Gombart
Linus Pauling Institute, Oregon State University

8:30-9:00 **Vitamin D requirements for optimum health**
Robert P. Heaney, Creighton University

9:00-9:30 **Vitamin D and the brain**
Thomas H. J. Burne, The University of Queensland, Australia

9:30-10:00 **Vitamin D and cancer prevention**
David Feldman, Stanford University School of Medicine

10:00 Coffee/Tea Break

HEALTH EFFECTS AND MECHANISMS OF ACTION OF XANTHOHUMOL

Chair: Fred Stevens
Linus Pauling Institute, Oregon State University

10:30-11:00 **Xanthohumol inhibits hyaluronan export and prevents osteoarthritis**
Peter Prehm, Muenster University Hospital, Germany

11:00-11:30 **Hops, xanthohumol and women's health**
Richard van Breemen, University of Illinois at Chicago

11:30-12:00 **Xanthohumol for treatment of metabolic syndrome**
Fred Stevens, Linus Pauling Institute, Oregon State Unversity

12:00-1:30 pm Lunch provided (LaSells Stewart Center)

DIET AND EPIGENETIC IMPACTS ON DISEASE AND AGING

Chairs: Rod Dashwood and Tory Hagen
Linus Pauling Institute, Oregon State University

1:30-2:00 **Bioactive food compounds and epigenetic mechanisms in breast cancer**
Donato Romagnolo, University of Arizona

2:00-2:30 **Methylation profiling in breast cancer prevention**
Clarissa Gerhäuser, German Cancer Research Center (DKFZ), Germany

2:30-3:00 **Modulation of microRNA profiles by dietary chemopreventive agents**
Alberto Izzotti, University of Genoa, Italy

3:00-3:30 Coffee/Tea Break

3:30-4:00 **Folic acid deficiency, aortic plaque formation and DNA methylation in vascular tissue**
Susan Duthie, University of Aberdeen, UK

4:00-4:30 **Nutritional influences on epigenetics and age-related disease**
Sang-Woon Choi, Tufts University

4:30-5:00 **TBA**

5:00-7:00 **Poster Session**
(hors d'oeuvres and beverages provided)

Friday, May 17

6:00 am Organized Walk/Run
7:30-8:30 Breakfast provided

HEALTH BENEFITS OF VITAMIN C: BEYOND SCURVY

Chair: Balz Frei
Linus Pauling Institute, Oregon State University

8:30-9:00 **Chemical biology of pharmacological ascorbate to treat cancer**
Garry Buettner, The University of Iowa

9:00-9:30 **Vitamin C supplementation effects on blood pressure**
Edgar R. Miller III, The Johns Hopkins University School of Medicine

9:30-10:00 **Vitamin C in brain development and cognitive function**
Fiona Harrison, Vanderbilt University

10:00 Coffee/Tea Break

MICRONUTRIENTS IN FERTILITY AND PREGNANCY

Chair: David E. Williams
Linus Pauling Institute, Oregon State University

10:30-11:00 **Folic acid: Fortification versus supplementation**
Helene McNulty, University of Ulster, Northern Ireland

11:00-11:30 **Role of micronutrients in human fertility**
Kelton Tremellen, Repromed, Australia

11:30-12:00 **Micronutrients and the developing brain**
Victoria Hall Moran, University of Central Lancashire, UK

12:00-1:05 pm Lunch provided (LaSells Stewart Center)

1:10-4:15 **Oral abstracts**

AWARD CEREMONY
Linus Pauling Institute Prize for Health Research

4:15 **Presentation by LPI Director, Balz Frei**
Plenary Lecture by Awardee

6:30 Reception
7:30 Banquet Dinner

Saturday, May 18

PUBLIC SESSION: WHOLE FOOD APPROACHES TO DISEASE PREVENTION

Co-sponsored by the Moore Family Center for Whole Grain Foods, Nutrition and Preventive Health, Oregon State University

Chairs: Emily Ho and Balz Frei
Linus Pauling Institute, Oregon State University

9:00-9:30 **Healthy dietary patterns in the prevention of breast cancer and metabolic syndrome**
Cynthia A. Thomson, University of Arizona

9:30-10:00 **Effects of whole grains and nuts on cardiometabolic syndrome risk factors**
Penny Kris-Etherton, Penn State University

10:00-10:30 **TBA**
Eric Rimm, Harvard School of Public Health

10:30-11:00 **An integrative, family-based approach to childhood obesity**
David Ludwig, Harvard University

11:00-12:00 **Panel discussion/Questions and answers**

Preliminary Program (as of October 26, 2012)



For more information about the Conference, please visit the LPI Web site at http://lpi.oregonstate.edu/conf2013 or phone the Institute at 541-737-5075

Continued from page 7 — Coenzyme Q_{10}

Hg, respectively. The randomized controlled trials included in this analysis used doses of 100-120 mg/day. In addition, several small, randomized, controlled trials have investigated whether coenzyme Q_{10} might improve vascular endothelial function (blood vessel dilation); the ability of blood vessels to relax or dilate is compromised in individuals with atherosclerosis, coronary artery disease, or diabetes. A 2011 meta-analysis examined the results of five such trials in those with type 2 diabetes mellitus, coronary artery disease, or endothelial dysfunction. Supplementation with coenzyme Q_{10} (150-300 mg/day for four to 12 weeks) was associated with a 1.7% increase in flow-dependent endothelial-mediated dilation, which may be clinically significant in terms of reducing risk for heart attack and stroke.

Because impaired mitochondrial function and oxidative stress contribute to the pathogenesis of neurodegenerative diseases, coenzyme Q_{10} has been studied as a potential therapy for Parkinson's disease, Huntington's disease, and Friedreich's ataxia. Compared to control subjects, studies have documented decreased ratios of reduced to oxidized coenzyme Q_{10} in platelets (irregularly shaped cell fragments that assist in blood clotting) and higher concentrations of oxidized coenzyme Q_{10} in cerebrospinal fluid of patients with Parkinson's disease. Additionally, one postmortem study found lower levels of total coenzyme Q_{10} in the cortex region of the brain compared to age- and sex-matched controls; however, no differences were observed in the other brain regions examined, including the *substantia nigra*—the site of neuron cell death in the brain stem of Parkinson's patients. While results of an early clinical trial of high-dose coenzyme Q_{10} (1,200 mg/day) were promising, more recent trials have generally not reported beneficial effects in patients with Parkinson's disease. In fact, a phase III trial of coenzyme Q_{10} supplementation (1,200-2,400 mg/day), in combination with vitamin E, was recently halted because it was deemed unlikely to be effective in treating Parkinson's disease.

Coenzyme Q_{10} has also been investigated as a potential treatment for inherited neurodegenerative diseases like Huntington's disease and Friedreich's ataxia. Huntington's disease is characterized by selective degeneration of striatal spiny neurons. Disease symptoms, which include movement disorders and impaired cognition, typically develop in the fourth decade of life and progressively deteriorate over time. Research in animal models of Huntington's disease implicates impaired mitochondrial function and glutamate-mediated neurotoxicity in disease pathogenesis. Some studies in mouse models of the disease have found that dietary coenzyme Q_{10} supplementation improves motor performance, reduces various hallmarks of Huntington's disease (i.e., brain atrophy, ventricular enlargement, striatal neuronal atrophy), and extends survival. Several studies in mice have also found that co-administration of coenzyme Q_{10} with remacemide (a drug that antagonizes the neuronal receptor activated by glutamate) results in greater improvements in most measured parameters. However, a 30-month randomized, placebo-controlled trial in 347 patients with early Huntington's disease found that coenzyme Q_{10} (600 mg/day)-racemide co-supplementation did not slow the functional decline associated with the disease. A phase III clinical trial administering a much higher dose of coenzyme Q_{10} (2,400 mg/day) or placebo to Huntington's disease patients is currently under way.

Coenzyme Q_{10} has also been investigated as a potential therapy for Friedreich's ataxia, an inherited neurodegenerative disease that causes iron accumulation within the mitochondria, resulting in increased oxidative stress and a decline in mitochondrial function. A pilot study in ten patients with the condition found that co-supplementation with coenzyme Q_{10} (200 mg/day) and vitamin E (2,100 IU/day) led to improvements in cardiac and skeletal muscle function and also helped to prevent the progressive declines in neurological function. Another study found decreased serum levels of coenzyme Q_{10} and vitamin E in patients with Friedreich's ataxia, suggesting that supplementation with both compounds may provide therapeutic benefit. Large-scale randomized clinical trials are needed to determine their efficacy in Friedreich's ataxia.

Although the utility of coenzyme Q_{10} supplementation for prevention or treatment of various diseases requires further study, even high doses of the compound appear to be relatively safe. There have been no reports of significant adverse side effects of oral coenzyme Q_{10} supplementation at doses as high as 1,200 mg/day for up to 16 months and 600 mg/day for up to 30 months. Recently, 1,200 mg/day was proposed as the observed safe level for coenzyme Q_{10} supplementation. Gastrointestinal symptoms, such as nausea, diarrhea, appetite suppression, heartburn, and abdominal discomfort, have been reported, but taking divided doses throughout the day might minimize these symptoms. The safety of high doses of coenzyme Q_{10} in pregnancy has not been established. However, use of coenzyme Q_{10} supplements may interfere with certain drugs. For example, concomitant use of warfarin (Coumadin) and coenzyme Q_{10} may decrease the anticoagulatory effect of warfarin. Additionally, HMG-CoA reductase inhibitors (statins) used to reduce cholesterol levels may also decrease biosynthesis of coenzyme Q_{10}, but it is not clear whether these drugs decrease coenzyme Q_{10} levels independent of a reduction in circulating lipids or decrease coenzyme Q_{10} concentrations in the body's tissues. For more information, please see the article on coenzyme Q_{10} in LPI's Micronutrient Information Center (lpi.oregonstate.edu/infocenter/othernuts/coq10). LPI

The Oxygen Club of California Meeting: Oxidants and Antioxidants in Biology

Stephen Lawson, LPI Administrative Officer

The Oxygen Club of California (OCC) held its biennial meeting in Alba, Italy, on June 20-23, co-sponsored, as usual, by the Linus Pauling Institute. Dr. Maret Traber, LPI's Helen P. Rumbel Professor for Micronutrient Research, is the President of OCC and served as one of the organizers of the meeting.

The meeting, entitled "Cell Signaling and Nutrient-Gene Interactions," featured 32 speakers—including three from LPI—and covered the following topics: "Nrf-2–driven Regulation of Antioxidant Defenses," "Nutrient-gene Interactions and Epigenetics," "Novel Roles of Micronutrients," "Lipid Oxidation and Signaling," and "Epigenetics, Metabolism, and Aging." These sessions were followed by a poster session with over 100 posters on relevant topics.

In his keynote lecture, Richard Weindruch noted that studies in rodents and monkeys have demonstrated that caloric restriction leads to an increase in healthspan—the portion of life free from chronic disease and pervasive decline in health and vitality. He and colleagues have been interested in understanding the molecular mechanisms that underlie the increase in healthspan in order to discover substances that could serve as mimics of caloric restriction because chronic caloric restriction is extremely difficult for people to accept. It's likely that substances that regulate energy metabolism may serve as such mimics.

Nrf2–driven Regulation of Antioxidant Defenses

Nrf2 is a transcription factor (a protein that regulates gene activity by binding to DNA) typically bound to another protein called KEAP in the cell's cytoplasm. Toxins and reactive oxygen molecules cause Nrf2 to dissociate from KEAP and migrate into the nucleus, where it induces antioxidant and detoxification genes that help protect cells. However, overexpression of Nrf2 may help cancer cells. Speakers noted that Nrf2 also attenuates inflammation and is induced by oxidized lipids and sulforaphane, a phytochemical in cruciferous vegetables like broccoli. Nrf2 has been found to be inhibited in umbilical cord cells in cases of pre-eclampsia and gestational diabetes, which may increase the risk of diabetes and heart disease in those infants when adults. Dietary selenium deficiency may result in increased Nrf2 activity as a compensatory mechanism.

Nutrient-gene Interactions and Epigenetics

Epigenetics is the study of changes in gene expression that don't change the underlying DNA sequence. Speakers discussed how chemical modifications of DNA, such as methylation, or of histones, which are proteins that DNA wraps around, affect gene expression. Acetylation (addition of acetyl chemical groups) of histones results in genes being turned on, and the deacetylation of histones causes genes to be turned off. Histone deacetylase (HDAC) inhibitors, therefore, keep genes, including tumor suppressor genes, active. In prostate cancer cells, the phytochemical HDAC inhibitor, sulforaphane, triggers apoptosis (programmed cell death). It was also reported that the B vitamin riboflavin lowers blood pressure in heart disease patients carrying a certain gene, which is prevalent in about 10% of people worldwide. Supplementation with riboflavin may significantly lower the risk for stroke in such people.

Novel Roles of Micronutrients

New roles for micronutrients and phytochemicals in health continuously emerge. Pomegranate juice has been shown to slow the increase in PSA in men after prostatectomy, and cell studies with pomegranate extract suggest that it is anti-inflammatory. Zinc has an important role in protecting the developing brain from oxidant injury

continued on page 12

Helmut Sies presented the Science and Humanity Award to José Viña of the University of Valencia, Spain (left to right: Maret Traber, Helmut Sies, José Viña)

Continued from page 11 - Oxygen Club of California

by several mechanisms, and zinc deficiency impairs the synthesis of the endogenous antioxidant glutathione. Studies on vitamin E and the risk for heart disease have been conflicting, but new evidence suggests that some patients, particularly diabetics with the haptoglobin 2-2 genotype, may benefit from supplementation. Coenzyme Q_{10} may help regulate fat metabolism; supplementation of obese mice lowers body weight and improves metabolic parameters. Lipoic acid and its derivatives induce cellular responses to stress caused by toxins and free radicals. Lipoic acid has also been found to inhibit atherosclerotic lesion development in a mouse model of atherosclerosis, as well as to decrease body weight gain and triglycerides. Through its effect on transcription factors NF-κB (implicated in inflammation) and Nrf2 (regulator of detoxification and antioxidant enzymes), lipoic acid reverses the decline in the antioxidant enzyme superoxide dismutase in the aortas of old rats. Using cigarette smoke as a source of oxidative stress, scientists showed that resveratrol found in red wine or curcumin from the spice turmeric (a major constituent of curry) inhibited inflammation in mice.

The Lester Packer Young Investigator Award was presented to Insa Ernst of the Christian-Albrechts University, Kiel, Germany (left to right: Maret Traber, Lester Packer, Insa Ernst)

Lipid Oxidation and Signaling

Oxidized fats in the body have a wide array of effects. While oxidized fats sometimes serve beneficial functions, they are associated mainly with inflammation and damage to proteins and other biomolecules. Oxidized fats that accumulate in atherosclerotic lesions may contribute to plaque instability and rupture, which could lead to heart attacks or strokes. Lipids in immune cells may control their function and response to inflammatory stimuli. Oxysterols are formed when cholesterol molecules become oxidized and are typically involved in pathological processes like atherosclerosis. In addition to serving as biomarkers of oxidative stress and inflammation, oxysterols help regulate lipid metabolism in the liver.

Epigenetics, Metabolism, and Aging

Aging is characterized by numerous declines in function and increased risk for major diseases. In old mice, Nrf2

Maret Traber presented the Aging Research Award to Claudio Franceschi of the University of Bologna, Italy (left to right: Lester Packer, Claudio Franceschi, Maret Traber)

activation and supplementation with nicotinamide, which is a derivative of the B vitamin, niacin, and the precursor for the NADH that donates electrons in chemical reactions, improved the survival of neurons and increased the levels of glutathione, an endogenous antioxidant. Oxidative stress and free radical damage have been associated with aging, but some studies correlate oxidative stress more closely with frailty in old age caused by declines in skeletal muscle function and motor neurons. Other studies implicate changes in sex hormones in age-related frailty. Proteasomes are cellular structures that degrade oxidized and damaged proteins. There is an age-related decline in the efficiency of proteasomes, leading to the aggregation of damaged proteins associated with the development of age-related diseases. LPI

(left to right: John Maguire, Maret Traber, Lester Packer, Angela Mastaloudis)

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

Dr. George B. Whatley
Ms. Helen P. Rumbel
Mr. Charles R. Lutz
Ms. Ella C. Brauch

Vitamin C, Lysine, and Lipoprotein(a) in Atherosclerosis and Angina Pectoris

Stephen Lawson, LPI Administrative Officer

Lipoprotein(a)

Although lipoprotein(a) [Lp(a)] was discovered about 50 years ago, surprisingly little is known about its cellular and biochemical functions. Lp(a) consists of a low-density lipoprotein (LDL) particle containing an apolipoprotein B-100 molecule linked to another apoprotein molecule called apo(a), which determines the functional characteristics of Lp(a) and largely accounts for its heart disease risk. Blood concentrations of Lp(a) are genetically determined and generally highest in blacks and lowest in whites. While concentrations of Lp(a) are stable in individuals over time, they can vary among people by 1,000-fold. There are many genetic variations in the size of the apo(a) particle in Lp(a), which accounts for the variable association with cardiovascular pathologies and events.

Many large-scale studies have explored the relationship between Lp(a) and cardiovascular diseases, consistently finding an association between high Lp(a) levels and atherosclerosis, heart attacks, or stroke; Lp(a) has been validated as a significant, independent risk factor for heart disease. Despite these significant correlations, the precise role of Lp(a) in elevating the risk for heart disease and stroke remains speculative. Lp(a) seems to be pro-inflammatory; it activates NF-κB, a transcription factor that binds to DNA and stimulates inflammatory processes. As a consequence, Lp(a) promotes the recruitment of immune cells called monocytes to the intima, the innermost layer of blood vessels and arteries facing the blood stream. Once monocytes have migrated to the intima, they can become engorged with oxidized LDL—becoming "foam" cells—and initiate atherosclerotic lesion formation.

Lp(a) also has been found to impair fibrinolysis, the process by which fibrin in blood clots is broken down by plasmin, the active form of plasminogen. By interfering with this process, Lp(a) contributes to thrombosis (blood clot formation) that could result in a heart attack or stroke. Clot formation in the arterial wall, called "mural thrombosis," contributes to the growth of atherosclerotic lesions; "occlusive thrombosis," on the other hand, blocks an artery and triggers a heart attack or stroke. Lp(a) may also enhance coagulation, which could further contribute to thrombotic events.

The Hypothesis

In 1990, Linus Pauling and a cardiologist colleague published a paper entitled "Hypothesis: Lipoprotein(a) is a surrogate for ascorbate" in the *Proceedings of the National Academy of Sciences* (*PNAS*) on the putative relationship between vitamin C and Lp(a). They hypothesized that Lp(a) serves as an evolutionary surrogate or substitute for vitamin C in animals that do not endogenously synthesize vitamin C, such as humans and monkeys. Indeed, Lp(a) is found in few species, mainly those that do not synthesize vitamin C. Since the absence of detectable Lp(a) in most species—almost all of which synthesize vitamin C—does not seem to be biologically disadvantageous, it was also proposed that vitamin C serves as a surrogate for Lp(a). In a subsequent paper, Pauling noted that our ancestors' loss of the ability to synthesize vitamin C and the acquisition of Lp(a) synthesis both occurred about 40 million years ago.

As a surrogate for vitamin C and owing to its pro-clotting functions, Pauling and his colleague suggested that Lp(a) might help repair lesions in the arterial wall caused by mechanical stress, free radicals, and/or sub-optimum collagen synthesis when vitamin C concentrations are insufficient. However, atherosclerosis results from the chronic, pathological deposition of Lp(a). A corollary is that adequate vitamin C status in humans, such as that achieved by sufficient intake, may help prevent arterial damage and reduce the need for the deposition of Lp(a) and/or lower its levels, thus preventing the development of atherosclerotic plaque. Scurvy caused by vitamin C deficiency results in capillary fragility and hemorrhage. Lp(a) may help by inhibiting fibrinolysis, thus preventing hemorrhage. Pauling and his colleague also suggested that the apo(a) part of Lp(a), rich in disulfide groups, may function as an antioxidant, thereby inhibiting LDL lipid oxidation associated with plaque formation. In this way, Lp(a) also would serve as a surrogate for the premier water-soluble antioxidant—vitamin C—when its levels are low.

In their first *PNAS* paper, Pauling and his colleague proposed that Lp(a) would be present in guinea pigs, a species unable to synthesize vitamin C. In a subsequent paper in *PNAS* in 1990, they showed that, indeed, Lp(a) is present in guinea pigs. They further found that vitamin C deficiency in these animals promoted the development of atherosclerotic plaque and that supplemental vitamin C prevented its development and the accumulation of Lp(a) in the arterial wall. They also suggested that depletion of vitamin C increases the permeability of the vascular wall, thereby contributing to the infiltration of Lp(a) and leading to plaque formation and mural thrombosis. In chronic vitamin C insufficiency, the prolonged action and accumulation of Lp(a) may result in the development of plaque (mural thrombosis) and adverse events like heart attacks and strokes related to occlusive thrombosis.

The *PNAS* papers were followed up with a series of papers published in the *Journal of Orthomolecular Medicine* (*JOM*) further outlining the putative relationship between vitamin C, Lp(a), heart disease, cancer, diabetes, and other diseases, emphasizing, especially, the role of chronic vitamin C deficiency in their etiology.

continued on page 14

Continued from page 13 — Vitamin C

Lowering Lp(a)

Unfortunately, neither diet nor exercise has been found to lower Lp(a) levels. There have been at least three randomized, controlled trials to evaluate the effect of supplemental vitamin C on Lp(a) levels. A 1994 study with 124 healthy men and women aged 17 to 74 detected no significant effect of 1 or 2 grams per day of vitamin C for one month on Lp(a) levels. Published in 1995, another study enrolled 44 patients with coronary heart disease who received either placebo or 4.5 grams per day of vitamin C for 12 weeks. The authors reported no statistically significant effect of vitamin C on Lp(a) levels. The last study, published in 2000, enrolled 101 healthy men and women who received either placebo or 1 gram per day of vitamin C for eight months. Again, the authors reported no significant effect of vitamin C on Lp(a) levels. These results conflict with an earlier uncontrolled, small study published in 1992 with 11 heart disease patients given 9 grams per day of vitamin C for 14 weeks. In that study, vitamin C lowered Lp(a) levels by an average of 27%. Overall, these studies suggest that supplemental vitamin C does not lower Lp(a) levels in humans.

However, a few strategies to lower Lp(a) levels have been suggested by some small studies. One study of 37 heart disease patients in 2002 found that daily doses of 81 mg of aspirin for six months lowered Lp(a) levels by an average of 82% in those subjects with high baseline levels of Lp(a) (>30 mg/dl). Another study in 1998 with 73 hypercholesterolemic patients reported that a daily dose of 2,000 mg of niacin for 96 weeks lowered Lp(a) levels by an average of 39%. This dose of niacin is almost 60 times higher than the tolerable upper intake level—the highest daily intake likely to pose no risk of adverse effects—of 35 mg/day for adults. The time-release niacin used in the 1998 study was generally well tolerated; common side effects included skin flushing and slightly elevated liver enzymes.

Other studies have reported that L-carnitine, a non-protein amino acid, lowers Lp(a) levels. One study in 2000 found that 2 grams per day of L-carnitine lowered Lp(a) levels by nearly 8% in 36 subjects with high baseline levels of Lp(a) (40-80 mg/dl). In 2003, a randomized, placebo-controlled study in 94 diabetic patients found that Lp(a) levels were lowered by 21% in patients taking 2 grams per day of L-carnitine for six months.

Lysine, Lp(a), and Angina

The amino acids lysine and proline are chemically converted to other forms (hydroxylysine and hydroxyproline, respectively) by vitamin C for collagen synthesis. In the case of chronic vitamin C deficiency, these conversions cannot take place effectively, and collagen synthesis is impaired, leading to the loss of the structural integrity of vascular and other tissues and subsequent hemorrhage, as previously mentioned. In this situation, fibrin, by promoting clot formation, may be valuable in protecting against blood loss. Pauling and his colleague speculated that Lp(a) binds excessively to fibrin in clots, impairing fibrin breakdown by plasmin. They also speculated that lysine may bind to Lp(a). In this case, lysine might help remove chronically deposited Lp(a) or interfere with its deposition. Thus, lysine may have therapeutic benefit in atherosclerosis and angina, which is caused by poor blood flow in arteries compromised by atherosclerosis.

Pauling published three case reports in the early 1990s in the *JOM* on the rapid relief from severe, exercise-induced angina in heart disease patients taking 3-6 grams/day each of lysine and vitamin C. There are some plausible explanations as to why vitamin C may have that effect, mainly related to its beneficial effect on endothelial function, i.e., its ability to relax blood vessels and improve blood flow via stabilization or increase of tetrahydrobiopterin—an enzyme cofactor that controls nitric oxide synthesis—but the role of lysine remains obscure. In two of the three case reports by Pauling, the patients had been taking large doses of vitamin C, but relief was only observed after the implementation of lysine. Because the relief from angina was so rapid in the reported cases, Pauling suggested, as previously mentioned, that lysine—by binding to Lp(a)—may quickly remove Lp(a) from plaque and also prevent its deposition in developing plaque. Unfortunately, no controlled clinical trials have yet been published to validate or refute this strategy to control angina, and the molecular interactions between lysine and Lp(a) aren't known.

Lysine is found in the diet, and it can be used in the body as a precursor to synthesize carnitine, an amino acid critical for mitochondrial energy production in cells. In his first case report on the amelioration of angina with vitamin C and lysine, Pauling noted that vitamin C is important in the hydroxylation reactions that synthesize carnitine from lysine. A number of studies have found that carnitine supplementation, as an adjunct to conventional therapy, is useful in treating heart disease, including heart attacks, heart failure, angina, and peripheral arterial disease.


The Commissioner of Patents and Trademarks

The United States of America

United States Patent

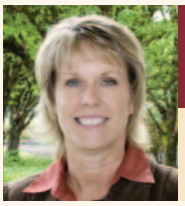
Patents

Several relevant patent applications were filed by Pauling and his collaborator, and three were awarded: "Use of ascorbate and tranexamic acid solution for organ and blood vessel treatment prior to transplantation" (1993), "Prevention and treatment of occlusive cardiovascular disease with ascorbate and substances that inhibit the binding of lipoprotein (A)" (1994), and "Therapeutic lysine salt composition and method of use" (1997).

The first of these described the treatment of organs or arteries used in transplantation with a solution containing vitamin C and tranexamic acid, both of which were postulated to inhibit the binding of Lp(a) to blood vessel walls, in order to prevent subsequent atherosclerotic complications. The inventors proposed that this method would have general applications for the prevention and treatment of heart disease, especially atherosclerosis, and utility for coronary bypass patients, diabetics, and patients undergoing hemodialysis or organ transplants. The patent awarded in 1994 explained the putative use of vitamin C, tranexamic acid, lysine, and nicotinic acid (niacin) to reduce plasma levels of Lp(a) and prevent its binding to the arterial wall, thereby inhibiting atherosclerosis. The final patent addressed the use of lysine, vitamin C, beta-carotene, *N*-acetyl cysteine (a precursor for the synthesis of glutathione, an important endogenous antioxidant), vitamin E, and nicotinic acid to prevent and treat heart disease. The evidence provided in support of the patent applications was based on experiments with guinea pigs and human aortas obtained postmortem. While animal models of human disease are frequently useful, they do not always predict human outcomes. No data from human clinical trials were presented, so the patent provisions remained largely speculative. It's not unusual for patents to be awarded in order for the inventor to establish priority in advance of actual proof.

Finally…

We know that vitamin C has value in promoting cardiovascular health. It not only reduces the risk for heart disease and stroke but also lowers blood pressure and promotes vasodilation in patients with atherosclerosis, thus improving blood flow that may also attenuate angina. To date, the hypothesized relationship between vitamin C and Lp(a) has been neither proven nor refuted. It's also not yet known if lysine itself, rather than serving simply as a precursor for carnitine synthesis, plays an important role in preventing or treating heart disease or angina. And neither the dose-response relationships nor various combinations of micronutrients have been adequately addressed experimentally. Controlled clinical trials are needed to answer these provocative questions. Lastly, there is no compelling evidence from controlled trials that any micronutrient or combination of micronutrients reverses atherosclerosis in humans, although some may inhibit its development or retard its progression. LPI



Developments

Michele Erickson
LPI Director of Development

All of us associated with the Linus Pauling Institute deeply appreciate your relationship with LPI and your deep admiration and fondness for our founder, Dr. Linus Pauling. It is my privilege to travel the country to meet many of you and hear your personal stories of inspiration, connection, and affection for Dr. Pauling. Often, I am asked, "Is there something meaningful I can do for the Institute that would honor Linus Pauling and his legacy?" With your encouragement, LPI is launching a new opportunity to do just that!

After much discussion with people who knew Dr. Pauling, followed his career, and were inspired by his mission to relieve human suffering, LPI identified two priorities for funding that will bring together your philanthropic intent and provide a meaningful way to honor Dr. Pauling. Those conversations helped us to focus on two areas of Dr. Pauling's expertise that were most meaningful to him: innovative, cutting-edge research and educating and mentoring promising new student scientists.

Our vision includes two endowed funds:

- The Linus Pauling Endowed Research Innovator Fund
- The Linus Pauling Endowed Fellowship Fund

The Linus Pauling Fellowship provides two years of support for a high-achieving graduate student in LPI. Each new Fellow will be selected and honored publicly at LPI's Diet and Optimum Health Conference, with special recognition of Dr. Pauling and his career as a mentor for young scientists. This enduring fellowship will be unique in that it not only supports full tuition and provides a stipend for the honored student but also provides a private curriculum experience involving the personal and scientific papers of Dr. Pauling. The curriculum includes a special emphasis on Dr. Pauling's interest and work in orthomolecular medicine and nutrition.

The Linus Pauling Endowed Research Innovator Fund will be awarded to a Principal Investigator in the Institute on a five-year rotating basis. These funds will support a faculty member's research that advances knowledge in areas that were of interest to Dr. Pauling in the last 25 years of his life. The Linus Pauling Research Innovator Fund encourages faculty investigators to pursue cutting-edge research and pilot projects that need initial support to establish new research directions. Such creative and innovative ideas lead to the breakthroughs we need most to advance the mission of the Linus Pauling Institute and the vision of Dr. Pauling.

To learn more about how your investment of funds, stocks, or a bequest can work with the gifts of other donors to create an opportunity for perpetual recognition and honor as a legacy for Dr. Pauling, please contact me at 800-354-7281 or michele.erickson@oregonstate.edu. I would love to hear YOUR story of Linus Pauling's impact on your life and share ideas on how you can be part of this profoundly meaningful opportunity to continue Dr. Pauling's mission to reduce suffering and improve human health for our world. LPI

Linus Pauling Institute

Stephen Lawson, Research Newsletter Editor
Oregon State University
307 Linus Pauling Science Center
Corvallis, Oregon 97331

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

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

OSU Oregon State University

GIVING to the Linus Pauling Institute

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

Micronutrient Research for Optimum Health

The Linus Pauling Science Center

Photo by Karl Maasdam

Look for these informative articles inside!

Page 1 ..... *From the Director*
Page 1 ..... *Healthy Aging Interview with Viviana Perez*
Page 6 .... *Briefly. . .*
Page 7 ..... *Coenzyme Q_{10}*
Page 8 ..... *Diet and Optimum Health Conference*
Page 11 .... *The Oxygen Club of California Meeting*
Page 13 .... *Vitamin C, Lysine, and Lipoprotein(a) in Atherosclerosis and Angina Pectoris*
Page 15 .... *Developments*

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