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The Linus Pauling Institute

RESEARCH NEWSLETTER

From the Director

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A study published recently in the medical journal *Archives of Internal Medicine*, which followed 161,808 women from the Women's Health Initiative over eight years, claimed to provide "convincing evidence that multivitamin use has little or no influence on the risk of common cancers, cardiovascular disease, or total mortality in postmenopausal women." This message was immediately sent around the world by the news media, leading people everywhere to believe that taking a daily multivitamin does no good and is a waste of money. I believe nothing could be further from the truth.

The study was an observational study, not a randomized controlled trial. As its name implies, an observational study "observes" what people do, what they eat, what dietary supplements they take, how they live, and what kind of diseases they develop. In contrast, randomized controlled trials take a group of subjects and randomly assign half of them to get a specific treatment, for example, a certain drug or vitamin, and the other half gets a placebo. After several years, researchers assess whether those who got the actual treatment developed less disease than those who got the placebo.

Every epidemiologist will tell you that observational studies cannot establish cause-and-effect relationships; they only can observe associations that can generate a new hypothesis, like "multivitamins might not lower risk of heart disease." The hypothesis then needs to be tested in randomized controlled trials to either prove it, establishing a cause-and-effect relationship, or refute it. Unless and until such trials have been conducted, one cannot draw any conclusions regarding causality, let alone make recommendations for the public.

Observational studies are limited because they are notoriously difficult to evaluate and interpret. For example,

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Vital Micronutrients In Depth

An interview with Balz Frei, Ph.D.
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Q. ***Your early research concerned the effects of toxins on mitochondria, the organelles within cells that produce energy. How do mitochondria respond to toxins?***

A. During my Ph.D. work in Switzerland, I used various toxins that generate oxidative stress, including a lipid hydroperoxide or oxidized lipid called *tert*-butylhydroperoxide and other toxins like menadione or alloxan, which undergo redox cycling and produce superoxide radicals and other reactive oxygen species, or ROS. I found that these ROS cause the release of calcium from mitochondria, which disrupts the calcium homeostasis in the cell. Calcium regulates many cellular processes, and its level in the cytoplasm is very carefully maintained. When ROS cause calcium release from mitochondria, normal cellular function becomes disrupted. I found that ROS cause chemical changes in a protein in the inner mitochondrial membrane, resulting in the opening of a calcium channel and release of calcium into the cytoplasm.

Q. ***You also did research on ubiquinol-10, or coenzyme Q10. What is coenzyme Q10, and what are its health effects?***

A. Coenzyme Q10 is an important part of the mitochondrial respiratory chain, which is a sequence of proteins involved in electron transfer leading to the synthesis of ATP, which is the cell's fuel. The reduced form of coenzyme Q10, called ubiquinol-10, also can function as an antioxidant. Like vitamin E, ubiquinol-10 or other ubiquinols can act as free radical scavengers and antioxidants in membranes. I found that ubiquinol-10 is the first line of defense against oxidation of human low-density lipoprotein, or "bad" cholesterol. When LDL is exposed to different types of oxidative stress, ubiquinol-10 is consumed

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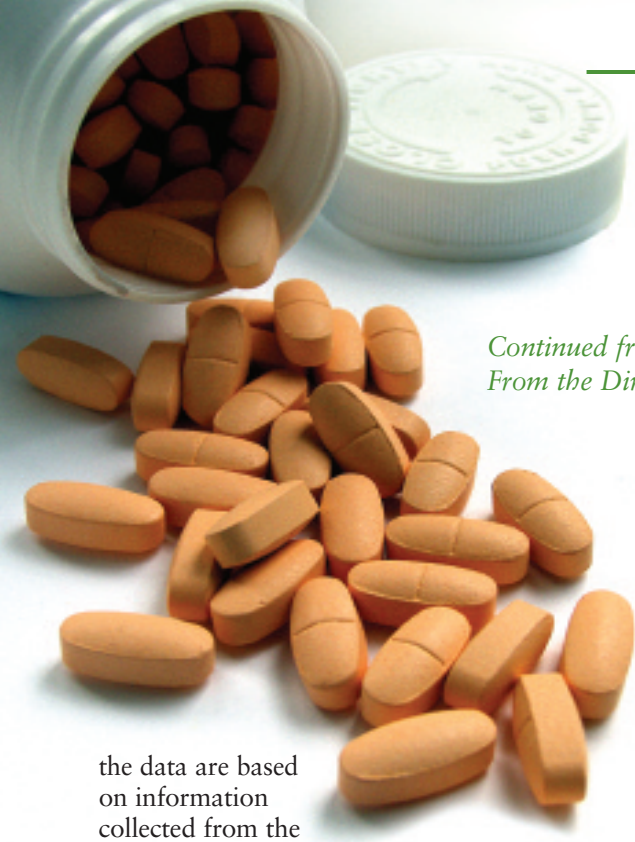

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the data are based on information collected from the participants, which is often selective and inaccurate (called "recall bias"). Behavior can change appreciably over eight years of observation. Multivitamin formulations vary considerably, and participants may have changed brands during the study. Most importantly, people who volunteer to be part of these studies are generally healthier than the average person—they are more health conscious, have a healthier diet, and exercise more, which can significantly affect the outcome of the study (called "healthy enrollee effect").

In the study, 41.5% of the women took multivitamins, and these multivitamin users were healthier than the non-users. Multivitamin users were more likely than non-users to be Caucasian, live in the Western U.S., drink moderate amounts of alcohol, smoke less, have a lower body mass index and a higher level of education, and report being physically more active and eating more fruit and vegetables and less fat. Each of these factors can strongly influence the multivitamin users' risk of disease, which makes it very difficult, if not impossible, to tease out the role of multivitamins alone. Epidemiologists use statistical models, in this case the "proportional hazards model," that they claim allows "adjusting" their data for all of these factors, but they often do not acknowledge that these statistical models are imperfect because they are based on many assumptions and are applied to incomplete and inaccurate data.

Here is the dizzying list of factors for which adjustments were made to the data in the study: "age; race/ethnicity; years since menopause (<5, 5-10, 10-15, and >15 years); body mass index; education; alcohol use; smoking; general health; history of bilateral oophorectomy [or ovariectomy, the surgical removal of one or both ovaries]; geographic region; physical activity; duration of prior postmenopausal estrogen therapy use (0, <5, 5-10, 10-15, and >15 years); duration of prior postmenopausal estrogen plus progesterone use (0, <5, 5-10, 10-15, and >15 years); fruit and vegetable intake; percentage of energy from fat; single supplements of vitamin C, E, or calcium and any other single supplement use and stratified according to age (5-year groups), and hormone therapy trial randomization assignment or study enrollment."

Because all of these adjustments were made using imperfect data and an imperfect statistical model, they are very unlikely to reveal the true effect of multivitamins. Furthermore, despite the statisticians' best efforts to take all of these "confounding" factors into consideration, there are numerous additional factors that haven't been discovered yet or were not measured in the study. This phenomenon is called "residual confounding" and is a major reason why observational studies can only generate hypotheses. In contrast, in randomized controlled trials subjects are randomly assigned to treatment or placebo, so all confounding factors, even the unknown ones, should be distributed equally between the two groups.

Given these considerations, it is inappropriate for the authors of the study to conclude that it provides "convincing evidence" that multivitamins have little or no effect on cancer or cardiovascular disease risk. The evidence is far from convincing; it is suggestive at best. In addition, while endpoints like cancer, heart disease, and death are important, it is possible, for example, that a daily multivitamin helps protect against other diseases, improves immune or brain function, or promotes general health. Also, eight years of multivitamin supplementation in women over 50 years of age, as assessed in the study, may be too little too late to have a significant effect. Obviously, the data do not apply to men because, among other things, they have a different risk profile for cardiovascular diseases and hormone-dependent cancers.

Most people in the U.S. have a poor diet and don't come close to consuming the recommended nine servings of fruit and vegetables every day. As a consequence, high percentages of the U.S. population do not even meet the recommended dietary allowances for many vitamins and essential minerals, including vitamins A, C, E, and K, folic acid, zinc, magnesium, and calcium. For example, data from the National Health and Nutrition Examination Survey indicate that over 90% of the population doesn't meet the recommended dietary intake for vitamin E, over 40% for vitamin A, 30% for vitamin C, and 50% for magnesium. And evidence is accumulating that most people in the U.S. are vitamin D deficient.

Given the reality that people will not improve their diet and often cannot afford to buy more fruit and vegetables, the next best thing and most cost-effective solution is to take a multivitamin. Despite the cynics' assertion that "popping vitamins is a waste of money," taking a daily multivitamin costs less than 10 cents a day. Even Dr. JoAnn Manson, a principal investigator of the Women's Health Initiative and co-author of the study, acknowledges that "the research doesn't mean multivitamins are useless. Multivitamins may still be useful as a form of [health] insurance for people with poor eating habits." And that's the large majority of the people in this country! LPI

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before any other antioxidants in LDL like vitamin E or beta-carotene. Ubiquinol-10's role is limited because it is present in small concentrations in LDL compared to vitamin E. Therefore, only a little bit of oxidative stress can completely oxidize all of the LDL's ubiquinol-10.

Q. Is coenzyme Q10 made in cells or do we get it dietarily?

A. About half is synthesized endogenously and half comes from the diet, mainly from meat, fish, and chicken. Vegetable oils also contain small amounts.

Q. Does much of a supplemental dose of coenzyme Q10 get into the blood stream?

A. Quite a lot gets into the blood stream, and some is incorporated into LDL. If LDL is taken up into the vascular wall and becomes part of an atherosclerotic plaque, coenzyme Q10 can be detected in the vascular wall at fairly high concentrations, usually correlated with the LDL concentration. But the uptake into cells and tissues is very limited.

Q. If coenzyme Q10 is an effective antioxidant, why is it found in atherosclerotic lesions?

A. Both the reduced and oxidized forms of coenzyme Q10 are found in human lesions. Ubiquinone-10 is the oxidized form of ubiquinol-10. The ratio of ubiquinol to ubiquinone tells you how much oxidation has occurred. In lesions, nearly all coenzyme Q10 is in its oxidized form because LDL is also oxidized.

Q. Are there any good studies that show health benefits from supplemental coenzyme Q10?

A. There are a number of studies by Karl Folkers at the University of Texas in Austin, who thought that coenzyme Q10 is very important in the prevention of heart failure, primarily through two mechanisms. It could improve ATP synthesis in mitochondria, which are typically dysfunctional in congestive heart failure, and it could protect against oxidative damage to the heart. While Folkers' papers did not convincingly show that coenzyme Q10 supplementation was protective against heart failure, the results looked promising.

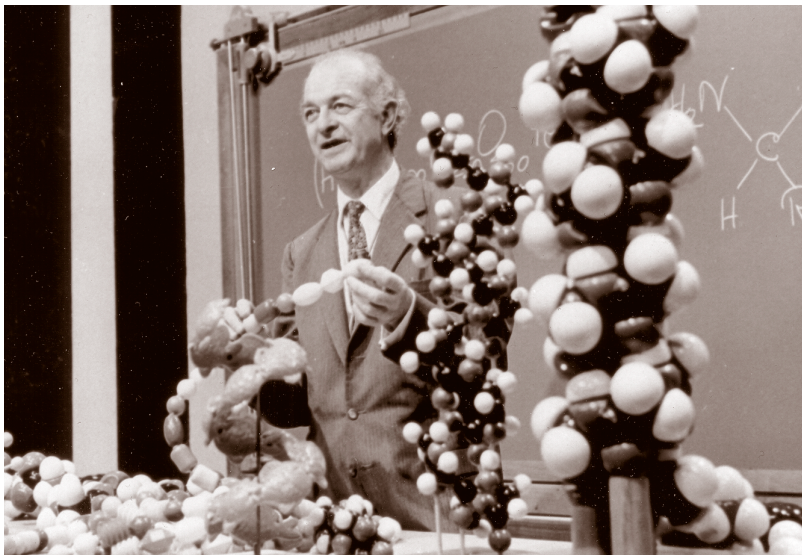
Q. Do you think that the interaction between coenzyme Q10 and vitamin E might be important in decreasing the risk of heart disease?

A. It certainly could. Again, the problem is that there is not much coenzyme Q10 in lipoproteins, so it has limited capacity to protect LDL from oxidation. My studies showed that ubiquinol-10 spares vitamin E, but—in contrast to vitamin E—is not recycled by vitamin C.

The synthesis of coenzyme Q10 occurs along the same biosynthetic pathway as cholesterol. A lot of people take statins to inhibit a specific enzyme in the cholesterol synthesis pathway with the goal to lower cholesterol levels. But that same enzyme also is required for the synthesis of coenzyme Q10. Some scientists, including Karl Folkers, have argued that it is important to take coenzyme Q10 supplements if you take statins in order to maintain normal coenzyme Q10 levels, which may be particularly important in heart disease patients.

Q. In 1989 you published a very influential paper on antioxidants that Linus Pauling called "striking". What did you find out about vitamin C?

A. That is my most cited paper—well over 1,000 times by other scientists in their publications—and was published when I was a post-doctoral fellow in Bruce Ames' laboratory at UC-Berkeley in the late 1980s. In that paper, I showed that vitamin C forms the first line of antioxidant defense in human plasma against many different types of oxidative stress. For example, I used superoxide radicals, peroxyl radicals, or hypochlorous acid—the same as bleach—which is an oxidant produced by white blood cells to kill invading bacteria and other pathogens. But hypochlorous acid can also damage the host tissues. Vitamin C is a very effective scavenger of hypochlorous acid, so if plasma is exposed to hypochlorous acid or to activated white blood cells, vitamin C forms the first line of antioxidant defense and inhibits oxidation of other susceptible targets in plasma. I was most impressed that I could not detect any oxidative damage to lipids in plasma as long as vitamin C was present. Only when vitamin C had been completely used up—or oxidized—was I able to detect oxidative damage. Other antioxidants in plasma, such as urate, which is a good water-soluble antioxidant, bilirubin, and alpha-tocopherol—vitamin E—the most abundant lipid-soluble antioxidant in plasma, were able to lower the rate of lipid peroxidation or oxidative damage, but unlike vitamin C, they were not able to completely protect against this kind of damage. My work highlighted the powerful role of vitamin C as an antioxidant in the body because vitamin C is found not only in plasma but also at high, millimolar concentrations in cells and tissues.



Q. Did you have a chance to meet Linus Pauling at that time?

A. Yes, I met Linus Pauling twice. The first time was at a conference at the National Cancer Institute in 1989 that highlighted the protective role of vitamin C in cancer and its underlying biochemical mechanisms, including anti-

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oxidant effects. The second time I met Dr. Pauling was at the Linus Pauling Institute of Science and Medicine in Palo Alto when I was invited to give a seminar with Bruce Ames on October 17, 1989. I'll never forget that day, not only because I had a chance to talk with Linus Pauling, but also because it was the day of the Loma Prieta earthquake in the Bay Area. When the earthquake hit, we were in your office, Steve, on Page Mill Road, and I remember leaving the building together and watching the undulating waves in the parking lot outside.

Q. It's well known that vitamin C reacts with certain metals like copper and iron in solution to generate reactive oxygen species like hydrogen peroxide and hydroxyl radicals. Paradoxically, vitamin C in the body, as you said, acts as an antioxidant. How can these contrasting activities be reconciled?

A. In the body, metal ions, such as copper and iron, are bound to specific proteins. For example, iron in blood is bound to transferrin, which is its transport protein. In cells, much of the iron is bound to ferritin, which is its storage protein. When metal ions are bound to proteins, ascorbate cannot usually interact with them—they are not accessible to ascorbate so free radical reactions don't occur. But these metal ions may come loose during pathological conditions, such as when a cell dies by necrosis and all its constituents rupture out. Under those conditions, ascorbate may interact with and reduce these free metal ions. The reduced metal ions could then react with oxygen to produce superoxide radicals. Superoxide radicals, in turn, can give rise to hydrogen peroxide and other reactive oxygen species, including hydroxyl radicals. It's only under pathological conditions when these metal ions are no longer attached to their proper binding proteins that vitamin C at physiological concentrations might act as a pro-oxidant.

Q. Didn't you do experiments in which you overloaded blood taken from volunteers with free iron, then added vitamin C, and did not find any detectable lipid oxidation?

A. That's correct. Even under extreme iron overload conditions in plasma, we could not see a pro-oxidant effect of vitamin C. That may be because the iron binds to plasma proteins other than transferrin, like albumin, which has binding sites for metals, and therefore the iron is sequestered from ascorbate. Even if metals are present and can interact with ascorbate, leading to free radical production, the resulting radicals and reactive oxygen species may in turn be scavenged by ascorbate. The overall result may be little or no oxidative damage. So the balance of pro-oxidant versus antioxidant activity of vitamin C may come into play under conditions of iron or copper overload. We also did some studies with iron-overloaded guinea pigs and, again, showed that the overall effect of ascorbate in those animals was antioxidant, not pro-oxidant.

Q. So the speculation that high-dose vitamin C supplementation might cause pro-oxidant effects in the body is not justified by the evidence?

A. No, it's not, except under pathological conditions with cell and tissue necrosis. The body has very tight mechanisms to keep vitamin C concentrations in a physiological range of about 30 to 100 micromolar in blood and one to five millimolar in cells. At those concentrations there is little if any danger of a pro-oxidant effect of vitamin C. However, Mark Levine at the NIH has found that in blood, very high—millimolar—concentrations achieved only by intravenous administration can lead to the production of hydrogen peroxide—a reactive oxygen species. His team's work implicates a metal-containing protein in this process that has yet to be identified. Dr. Levine is pursuing the idea that intravenous vitamin C, in contrast to oral vitamin C, may have benefit in cancer therapy through this mechanism of local hydrogen peroxide production and selective killing of cancer cells.

Q. When you were at the Whitaker Cardiovascular Institute at Boston University School of Medicine, you worked on vitamin C's effect on endothelial cells, which are the cells that line the blood vessels and arteries. How does vitamin C affect vascular function?

A. Vascular function is regulated mainly by a molecule called nitric oxide, which is produced in the endothelial cells that line the arteries. Endothelial cells contain an enzyme called endothelial nitric oxide synthase. When this enzyme produces nitric oxide, the blood vessels dilate. Nitric oxide was originally called endothelium-derived relaxing factor or EDRF, and the discovery of nitric oxide as EDRF led to a Nobel Prize in Medicine in 1998. Superoxide, an oxygen radical whose existence was postulated in the 1930s by Linus Pauling, can also be produced in the arterial wall. It inactivates nitric oxide and reacts with it to form another compound called peroxynitrite, which is a nasty chemical that can cause oxidative and "nitrative" damage. Ascorbate has been postulated to scavenge superoxide radicals, thereby preserving nitric oxide and its vasodilatory effects. We tested that idea by giving vitamin C to patients with heart disease who had impaired blood vessel relaxation. We were able to show that oral ascorbate supplementation was very effective in improving, and even normalizing, vasodilation in many patients. Subsequent work by my colleague, John Keaney, at Boston University found that ascorbate cannot preserve nitric oxide action by scavenging superoxide because reactions between superoxide and nitric oxide happen too fast for ascorbate to intervene. Instead, Dr. Keaney found that ascorbate is involved in the regeneration of an important co-factor for endothelial nitric oxide synthase called tetrahydrobiopterin. Tetrahydrobiopterin gets oxidized during enzyme activity and is reduced back to its active form by vitamin C to serve as a co-factor in the enzyme's reaction.

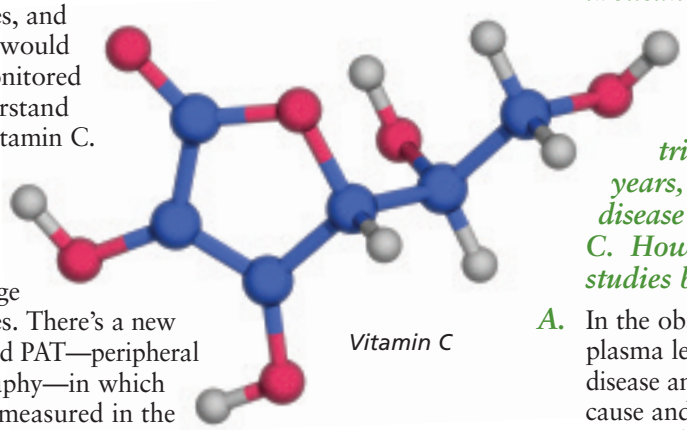
Q. Is nitric oxide activity impaired in people with atherosclerosis?

A. Yes, nitric oxide synthesis is impaired in many patients with risk factors for atherosclerosis and heart disease. For example, patients with high cholesterol level—hypocholesterolemics—are known to have decreased nitric oxide synthesis and impaired vascular function. The same is true for diabetics, smokers, obese people,

and patients with hypertension. There have been dozens of clinical studies with people with impaired vasodilation that have found oral or intravenous vitamin C to provide significant benefit. This is a very consistent finding, probably one of the most consistent results in any clinical setting for a micronutrient or vitamin. It astounds me that this has not been incorporated more into clinical practice. We know that vasodilation is linked to risk for heart disease and, in particular, angina, which is essentially caused by vasoconstriction. So, impaired vasodilation is a risk factor for heart disease, and I think it would be a good strategy to give vitamin C to patients with risk factors for atherosclerosis to help normalize their blood vessel function.

Q. ***A number of clinical trials have shown that supplemental vitamin C in doses of 500 mg or 2,000 mg lowers blood pressure by about 10 points in people who are mildly hypertensive. Is that due to the vasodilatory effect?***

A. It very well could be. Vasodilaton is usually measured using ultrasound in the conduit arteries like the brachial artery. But blood pressure builds up in the capillaries, not conduit arteries, and the capillaries would have to be monitored to better understand the effect of vitamin C. That has been done to some degree, but most of the data are in large conduit arteries. There's a new technique called PAT—peripheral artery tomography—in which blood flow is measured in the finger tips where microvessels are located. These studies have shown beneficial effects of vitamin C and some other dietary compounds, such as flavonoids from cocoa or tea.



Vitamin C

Q. ***Does vitamin C help prevent atherosclerosis?***

A. That's still controversial. Our studies have shown convincingly that vitamin C is an excellent antioxidant that protects against LDL oxidation and improves endothelial nitric oxide production, which has important implications for atherosclerosis and heart disease. But overall, there is not sufficient evidence from human studies to conclude that vitamin C can protect against atherosclerotic lesion development.

Q. ***What about lesion stability?***

A. In that regard, vitamin C's role in collagen synthesis becomes an important consideration. For example, there have been studies with mice that cannot synthesize their own vitamin C because they have an enzyme knocked out that is required for vitamin C biosynthesis. Humans lack that same enzyme, L-gulonolactone oxidase. Those vitamin C-deficient mice have severely impaired collagen synthesis and develop more atherosclerosis with unstable plaques that would make them prone to heart attacks and strokes.

Q. ***How do the effects of tea on endothelial function compare to those of vitamin C?***

A. Tea and vitamin C have similar effects in improving or normalizing vascular function. Tea probably acts through a different mechanism—not through regeneration of tetrahydrobiopterin like vitamin C—but through modification of the enzyme endothelial nitric oxide synthase. My colleague John Keaney showed that black tea polyphenols can modify the estrogen receptor-alpha, which triggers a complex cascade of signaling events inside the cell that eventually modify endothelial nitric oxide synthase by phosphorylation, which, in turn, increases its activity. So vitamin C and tea could act synergistically, one by recycling an essential co-factor and the other by chemically modifying the enzyme.

Q. ***Many observational epidemiological studies have found an inverse relationship between vitamin C intake and the risk for heart disease, such as the Nurses' Health Study and the Physicians' Health Study, both of which were long-term studies, 10 years and 8 years, respectively, with tens of thousands of subjects. Another long-term, large-scale study found that those with the highest plasma levels of vitamin C had a 40% lower risk of stroke. Yet the recent Physicians' Health Study, a clinical trial of over 14,000 men followed for eight years, found that there was no effect on heart disease risk in men taking 500 mg/day of vitamin C. How can these results from the observational studies be reconciled with this clinical trial?***

A. In the observational studies, you look at correlations like plasma levels of vitamin C and a lower incidence of heart disease and stroke. Those kinds of studies cannot establish cause and effect, but lead to hypotheses that can be tested in a randomized clinical trial. Some of the studies looked not only at dietary vitamin C intake but also at supplemental vitamin C. For example, in the Nurses' Health Study, supplementation was associated with a decreased incidence of cardiovascular disease. That's pretty good evidence that it is vitamin C itself and not simply fruit and vegetable intake that was responsible for the decreased risk for heart disease. It's fairly easy and straightforward to determine vitamin C intake from supplements because people know whether they took vitamin C supplements. Dietary vitamin C intake is assessed with food frequency questionnaires, which are notoriously imprecise. Overall, as you said, the evidence from observational studies is very strong for both dietary and supplemental vitamin C. The English Norfolk Study measured plasma levels of vitamin C in thousands of subjects, which is the best way to determine vitamin C status in the body, rather than trying to assess dietary or supplemental intake. That study has yielded some amazing findings for vitamin C, showing strong inverse associations between plasma vitamin C levels and overall mortality or mortality from heart disease and cancer. Again, that's an association, not cause and effect. Plasma vitamin C could simply be a

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marker for fruit or vegetable intake. Because causal relationships cannot be established by observational studies, it's important to conduct clinical trials.

Clinical trials divide subjects into two subgroups; one gets a placebo and the other one gets vitamin C. At the end of the study period, the incidence of heart disease or cancer or other chronic diseases in the groups is compared. In this kind of a trial the researchers can randomize, or equally distribute, unknown risk factors among the groups, making it a much better design than observational studies. But clinical trials also have serious limitations, especially when studying micronutrients. For example, most clinical trials are done in patients with existing disease, so those are secondary prevention or treatment studies, not primary prevention of disease in healthy people. The role of vitamin C in disease treatment may be very different from the role of vitamin C in disease prevention. However, it is cost-prohibitive to do these primary prevention studies because one would have to supplement and observe the subjects for over 20 years, starting at an early age, to truly assess the preventative effects. The secondary prevention or treatment studies are done for a few years in high risk patients who often already have the disease. Intervention late in the disease process for a few years may not work because it's just too little too late. Another limitation is that vitamin C is present in these subjects at fairly high baseline levels; otherwise they would suffer or have died from scurvy! Therefore, the statistical power of the study is quite low because vitamin C body status is not zero and can only be increased to a limited degree. In studies on drugs, on the other hand, the subjects either have the drug in their bodies or they don't, so there is a true placebo, unlike studies with vitamin C. Yet another limitation is that the subjects are not stratified or enrolled in the study according to their level of oxidative stress. If the hypothesis being tested is that vitamin C reduces oxidative damage, leading to a reduction in the risk for heart disease, for example, then one needs to know which subjects have elevated oxidative stress. If oxidative stress or oxidative damage is not measured in these subjects at the beginning of the study, we don't really know who would benefit the most from vitamin C supplementation. It's like doing a study with an anti-hypertensive drug without measuring blood pressure in your patients. The pool of patients who might benefit is diluted if everybody is included irrespective of oxidative stress status, further lowering the statistical power of the study. These kinds of limitations often get no attention but may explain why observational studies have shown promising results for vitamin C, whereas some clinical trials have not.

Q. Heart disease and cancer have been linked to oxidative stress, as well as chronic inflammation. Does vitamin C reduce inflammation?

A. We didn't see direct anti-inflammatory effects of vitamin C in our studies. For example, we exposed endothelial cells, the cells that line the artery, to certain inflammatory cytokines, which caused the cells to become inflamed, but loading these cells with vitamin C had no effect. It may be that vitamin C could have indirect anti-inflammatory effects through increased nitric oxide production, because nitric oxide has anti-inflammatory effects. Some recent evidence suggests that vitamin C in white blood cells, which get activated in response to injury, is important in programmed cell death of these cells. Without adequate vitamin C in these inflammatory cells, they remain active after they have done their job killing the bacteria or pathogens, which could lead to chronic inflammation. By helping to eliminate white blood cells when they are no longer needed, vitamin C may have an overall anti-inflammatory effect. This is important because chronic inflammation has been linked to many chronic diseases.

We have been very interested in some other anti-inflammatory dietary factors, particularly lipoic acid, which is a short fatty acid with two sulfur-containing groups that affects lipid and glucose metabolism and enhances the body's detoxification mechanisms. We exposed endothelial cells to inflammatory cytokines and observed convincing anti-inflammatory effects. Since chronic inflammation has been linked to atherosclerosis, lipoic acid may have important beneficial effects through its anti-inflammatory and metabolic effects. Indeed, we showed that lipoic acid supplementation lowers vascular inflammation and inhibits atherosclerosis in experimental mouse models.

Q. Dr. Gladys Block, a former trustee of the Linus Pauling Institute of Science and Medicine and a professor at UC-Berkeley, showed that supplemental vitamin C was effective in lowering inflammation, as measured by C-reactive protein, or CRP, in blood, mainly in people who had elevated levels of inflammation but not in people with normal baseline levels. Do you think that this might help explain why some of the clinical trials with vitamin C have been a bit ambiguous?

A. CRP is an independent risk factor for heart disease, so that's an important finding by Dr. Block. In order to see decreased inflammation, one has to treat subjects with elevated levels of inflammation. If subjects are enrolled in a clinical trial with normal levels of inflammation, the overall patient pool may be too diluted to see an effect of vitamin C. Dr. Block also found that vitamin C lowered F_2-isoprostanes, which are markers of oxidative stress, but again only in subjects who had abnormally elevated levels of F_2-isoprostanes. It all makes sense—you can only normalize what's abnormal. That's a crucial issue in the design of clinical trials that is not given nearly enough attention.

Q. The DRI—dietary reference intakes—for vitamin C were set by the Institute of Medicine in 2000 with an RDA for adult men of 90 mg/day and for adult women of 75 mg/day. The tolerable upper level of intake, or UL, was set at 2,000 mg/day. What do you think of those recommendations?

A. I think both the RDA and UL are too low. Levels that the Institute of Medicine recommends for the RDA are still, first and foremost, based on the prevention of scurvy, although they took some additional biological effects of vitamin C into account. We recommend at least 400 mg/day because that's the level that, in healthy people, produces maximal vitamin C levels in plasma and cells and, therefore, tissues as well. We think that maximizing

body vitamin C levels is beneficial because there will be better antioxidant protection and potentially better protection against some chronic diseases. That is not achieved with 90 mg in men or 75 mg in women. Studies by Dr. Mark Levine at the NIH have shown that 400 mg per day is required to fully saturate the body with vitamin C in young, healthy individuals.

If you get sick or have a chronic condition or are older, your vitamin C requirements may very well be higher than 400 mg per day in order to reach tissue saturation. So taking 500 mg, 1,000 mg, or even more per day will ensure tissue saturation and certainly has no adverse health effects. The tolerable upper intake level for vitamin C—2,000 mg per day—is based on a potential side effect of gastrointestinal disturbances and diarrhea. That is really a minor side effect and easily corrected by decreasing intake. The data that the Institute of Medicine used to determine the UL for vitamin C are very limited. Some people don't experience any gastrointestinal symptoms even at much higher doses of vitamin C. The Institute of Medicine couldn't identify any serious side effects, only anecdotal reports of rebound scurvy or kidney stones that are not strong enough to set a definitive UL. Another consequence of setting the UL for vitamin C at two grams has been that clinical studies using much higher levels of vitamin C seem to have problems being approved when reviewed by local Institutional Review Boards because they are concerned about "toxic" effects. The Institute of Medicine did note that studies should not be discouraged based on the UL and that their recommendations are for the general population. Nevertheless, it certainly sends the wrong signal to set a UL of two grams because much higher doses of vitamin C are completely benign.

Q. ***There are a lot of different types of vitamin C in the marketplace, including mineral ascorbates, vitamin C with bioflavonoids, vitamin C with metabolites, and ascorbyl palmitate. What form of vitamin C is the best to take?***

A. Just plain vitamin C. The salt—sodium ascorbate—may be better for some people with sensitive stomachs because of its neutral pH. You may want to choose a potassium or a calcium salt, but I don't think it's necessary to take any other preparations. Some products contain breakdown or oxidation products of vitamin C that have no known health benefits. Most experimental and clinical studies have used either ascorbic acid or sodium ascorbate.

Q. ***Is vitamin C valuable in treating cancer?***

A. As we discussed earlier, there is great interest now in intravenous vitamin C as adjunctive cancer therapy, an issue that was dear to Linus Pauling. His studies in the early 1970s with Ewan Cameron were done with oral and intravenous vitamin C and showed very good effects on quality of life and survival in terminal cancer patients. Those results could not be confirmed in the Mayo Clinic studies in the 1970s and 1980s, but they used only oral vitamin C. Mark Levine picked up on this difference between the Pauling-Cameron studies using intravenous vitamin C and the Mayo Clinic studies using oral vitamin C. He had observed in his early pharmacokinetic studies that much higher vitamin C concentrations can be achieved in blood after intravenous administration compared to oral vitamin C. Dr. Levine and his colleagues have shown that vitamin C given intravenously can produce hydrogen peroxide at the site of the tumor and lead to the selective killing of cancer cells. Vitamin C given orally probably won't produce concentrations high enough to generate hydrogen peroxide and kill cancer cells. Now we have a mechanistic understanding of how these large doses of vitamin C might work to be useful in cancer therapy, and Dr. Levine is pursuing these ideas in clinical studies. We are, of course, very excited about these studies because they may lead to a simple, effective treatment for certain types of cancer. I'm sure Linus Pauling would be pleased.

Q. ***In the early 1990s the Harvard epidemiology group reported that vitamin E intake was associated with protection from heart disease, but then follow-up studies seemed to show either no effect or perhaps detrimental effects. What have we learned from the controversies surrounding the use of supplemental vitamins C and E?***

A. We have learned that these clinical trials should not be considered the gold standard. If something doesn't work in a clinical trial, it will be dismissed, not just for that particular application, but generally. For example, when supplemental beta-carotene was shown to further increase the risk of lung cancer in smokers, many physicians dismissed all antioxidant supplements as worthless or even potentially dangerous. But it's much more complicated than that. In fact, beta-carotene is not a good antioxidant. It can scavenge singlet oxygen, but this type of reactive oxygen species may only play a role in skin or eye diseases. On the other hand, vitamin C is a water-soluble antioxidant that scavenges many different reactive oxygen species, and vitamin E is an effective lipid-soluble antioxidant in membranes and lipoproteins. Those vitamins have different biological effects because they have different mechanisms and chemical properties. One cannot dismiss all antioxidants if one of them doesn't seem to give the expected result in a clinical trial. To properly evaluate them, one needs to look at the totality of scientific evidence, including cell culture and animal studies that help establish biological mechanisms and plausibility. Animal studies are very valuable for this purpose because they are kept in the same controlled environment and

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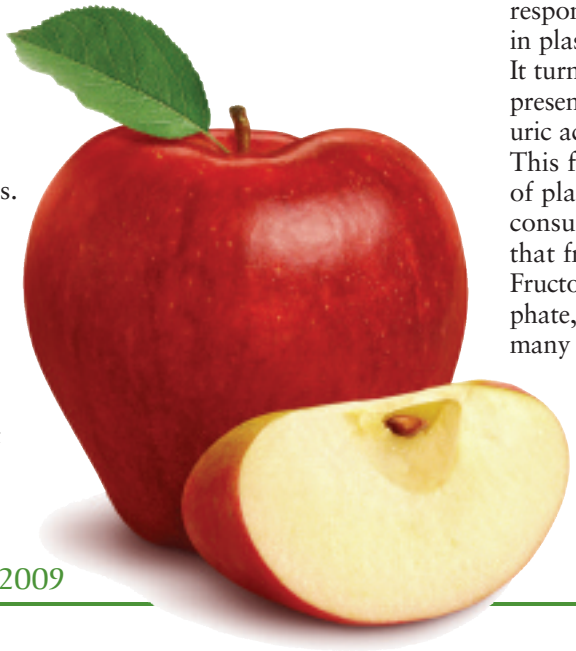
have the same genetic background. All the animals in a study are treated exactly the same, which increases the probability of seeing an effect, even if it is small. It's much easier to see an outcome for vitamin C or vitamin E in animal studies than in human studies, where there are a lot of genetic polymorphisms, different lifestyles, dietary variations, and many more confounding variables. That's why we should not overemphasize human studies but look at the totality of the evidence. If you do that for vitamin C or vitamin E, you can still make a very strong case that they have important roles in biology and human health above and beyond their role in preventing deficiency disease. Another lesson we've learned is that many of the clinical trials may have used doses that are too low or given for an insufficient length of time to see an effect. Again, these are chronic diseases—heart disease, cancer, Alzheimer's, or other neurodegenerative diseases—that usually develop over decades. Intervening with a relatively low dose for a couple of years may be too little too late. Even if you find a positive result in a particular study group, you can't generalize to the population at large. People who enroll in these studies are, to begin with, often different from the average person. They have an interest in their health, are usually more educated, and are more disciplined—they adhere to the treatment that they are given. This is called the "healthy enrollee effect," which means the people being studied are healthier than average and have a lower risk of chronic disease. That's another important limitation of clinical trials.

Q. Rather than worrying about people getting too much vitamin C or vitamin E, don't you think it's a bigger public health problem that many Americans get too little of these vital micronutrients?

A. Absolutely! And that's not only true for vitamin C and vitamin E but also for many other micronutrients, including some essential minerals like zinc, which is crucial for many biological processes. The focus in the media about potential adverse effects of supplements is really distracting from a much more important problem that we have in this country—people eat a very unhealthful diet, not enough fruit and vegetables, and, as a consequence, often don't even get the RDA of most vitamins and minerals. A very simple and straightforward way to mitigate that situation is to recommend multi-vitamins/minerals to everybody, so that everyone gets at least the recommended dietary allowance.



Q. Many people believe that flavonoids in fruit and vegetables are potent antioxidants. It's easy to find rankings of fruits, such as prunes and berries, based on their ability to lower oxidation in the test tube. Do you agree that flavonoids have strong antioxidant functions?

A. In the test tube, yes, but not in the human body. In test tube or *in vitro* experiments, flavonoids do pretty well as antioxidants, especially if one calculates the number of free radicals that each flavonoid molecule can scavenge. These are large molecules with lots of hydroxyl groups, so it makes sense that they would be able to scavenge a lot of free radicals. But if you look at their reaction rates with free radicals, they are not nearly as effective as vitamin C. Each vitamin C molecule can only scavenge two free radicals, so quantitatively vitamin C might not be as good as flavonoids, but qualitatively it's much better. More importantly, in the body flavonoids are not present at nearly the same concentrations as vitamin C because their absorption into blood from the gastrointestinal tract is very limited. For example, flavonoids may be found in plasma in concentrations in the submicromolar range, whereas vitamin C may be present in concentrations of about 50 micromolar. That tiny flavonoid concentration cannot make a significant contribution to antioxidant protection. In cells, the situation is even more extreme—vitamin C is present at a concentration of one to five millimolar. That's at least 40 times greater than its plasma concentration. The concentration of flavonoids in cells is still only in the micromolar range, and it's impossible for them to significantly contribute to free radical scavenging in cells.

Q. You studied the antioxidant capacity of flavonoids in apples. What did you find?

A. We wanted to find out if flavonoids from apples can make an important contribution to antioxidant activity in plasma. We fed apples or bagels, which have no flavonoids, to volunteers and then measured how the antioxidant capacity of their plasma changed over time. To our surprise, the antioxidant capacity of plasma increased quite substantially after apple consumption. As we expected, bagels had no effect. Other people have made similar observations and concluded that the flavonoids in fruit or vegetables were responsible. We went further and tried to identify what in plasma caused the increased antioxidant protection. It turned out not to be the flavonoids—again, they were present at only very small concentrations—but was instead uric acid that increased in plasma after apple consumption. This fully explained the increased antioxidant capacity of plasma. Why does uric acid increase after apple consumption? Scientists have known for over 40 years that fructose, a fruit sugar, increases uric acid production. Fructose causes the breakdown of adenosine monophosphate, a nucleotide in the liver, to uric acid. Apples and many other fruits contain lots of fructose, and eating them leads to the plasma increase in uric acid, which is a strong antioxidant.

Q. How did you confirm that relationship?

A. We had the same subjects drink a fructose solution, which resulted in the same increase in uric acid levels and antioxidant capacity of plasma as eating apples.

Q. *If flavonoids are not responsible for antioxidant activity in the body, what are their health benefits?*

A. As I explained, they are present in very small concentrations in plasma and cells, so there must be mechanisms that are triggered by these low concentrations. For example, hormones in the body are also present in very small concentrations. They react with receptors, which then send out signals inside cells that produce a biological effect, even though you start out with a very small concentration of the effector molecule. I think the same is true with flavonoids. They bind to proteins, and if the protein happens to be a receptor and triggers a cell-signaling cascade, you may end up with a significant biological effect. For example, black tea has a lot of polyphenols or flavonoids called catechins. They have been shown to activate the estrogen receptor alpha, which then modifies endothelial nitric oxide synthase, increasing the enzyme's activity to produce nitric oxide. I think that most flavonoids act by increasing cell signaling, activating enzymes, or increasing the activity of transcription factors, leading to upregulation of certain proteins.

Q. *In recent years you have become interested in lipoic acid. Why is lipoic acid important in health?*

A. The main biological function of lipoic acid is to act as a co-factor for a couple of enzymes in mitochondria that are crucial for energy metabolism. At high concentrations, lipoic acid can have many additional effects that go above and beyond its role in mitochondrial energy metabolism. One of those effects may be to act as an antioxidant. However, similar to the flavonoid story, I do not think that lipoic acid—or more accurately, its reduced form—can directly act as a free radical scavenger in the body. Instead, lipoic acid can have an antioxidant effect by upregulating certain enzymes that either participate in the synthesis of antioxidants, like glutathione, or are antioxidants themselves, like superoxide dismutase. Lipoic acid also improves glucose utilization and has anti-inflammatory effects that may protect against atherosclerosis and diabetes. It has also been shown that lipoic acid can reduce appetite by acting on a brain region called the hypothalamus. When mice are fed lipoic acid, they gain less weight than control mice not given lipoic acid. Lipoic acid may also have an effect on energy metabolism beyond the role it plays in mitochondria. In particular, it may affect the synthesis and utilization of fatty acids in a way that increases energy metabolism.

Q. *How does lipoic acid affect vitamin C status?*

A. There is some preliminary evidence from Tory Hagen's lab in LPI that lipoic acid can upregulate the sodium-dependent vitamin C transporter, which is the protein that transports vitamin C across the cell membrane into cells.

Q. *Does lipoic acid act in the early stages of atherosclerotic lesion development?*

A. We found that feeding lipoic acid to mice that have high cholesterol levels due to a genetic deficiency inhibited atherosclerosis. It would be interesting to find out whether lipoic acid can stop the progression of existing atherosclerosis or even reduce lesion size after they have already formed.

Q. *Some animal studies have shown that lipoic acid supplementation improves cognitive function and memory. Why?*

A. Research has indicated that lipoic acid may positively affect the hippocampus, a brain region directly associated with memory. In conjunction, Tory's work has shown that lipoic acid limits oxidative damage and improves mitochondrial function, which is critical for normal brain function. As we age, our mitochondria become less efficient in converting fat into energy. The mitochondrial membrane potential declines and less ATP is made. When Tory fed lipoic acid or lipoic acid in combination with acetyl-L-carnitine to old rats, the mitochondria became more efficient and generated more ATP. The mitochondrial membrane potential went back up almost to the level seen in young animals.

Based on our studies using endothelial cells and mice that develop atherosclerosis, we are now embarking on a clinical study to see whether those laboratory findings can be reproduced in humans. In collaboration with Oregon Health & Science University and supported by a grant from the National Institutes of Health, we are giving lipoic acid to two patient groups. One group consists of subjects who are overweight or obese and have high plasma triglyceride levels. We want to see if lipoic acid supplementation can reduce weight, improve body composition in terms of fat versus muscle mass, and lower triglyceride levels—effects we saw in mice. We will also examine anti-inflammatory effects by measuring markers of inflammation in blood, including CRP, and antioxidant effects by measuring F_2-isoprostanes. We are recruiting patients into that study now. In the second study, which will be initiated in about a year, we will investigate the effect of lipoic acid in patients with heart disease. Only patients with increased inflammation and increased oxidative stress will be enrolled because we don't want to repeat the mistake of mixing together subjects with normal and elevated levels of inflammation and oxidative stress, as was done in other clinical trials. We want to see whether those patients who have increased oxidative stress and inflammation benefit from lipoic acid supplementation. We will also measure triglyceride levels and check body weight and composition.

Q. *What dose and form of lipoic acid will you use in those studies?*

A. We are using the *R* form of lipoic acid, which is the natural form. The *S* form is synthetic, and the *R* form may have better biological activity. Most commercial supplements are a mixture of half *S* and half *R* forms. The dose in our study is 600 mg a day, given in the morning on an empty stomach in the form of two pills of 300 mg each.

Q. *Have any side effects or toxicity been reported for high doses of lipoic acid?*

A. Lipoic acid is known to be an insulin mimetic, so it can stimulate glucose uptake, and some people may experience transient hypoglycemia. The glucose levels may drop too rapidly in their blood stream, which could cause dizziness or vertigo.

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However, lipoic acid is generally recognized as safe. It is approved in Europe for the treatment of diabetic neuropathies, where it's even given intravenously.

Q. What about the pharmacokinetics of lipoic acid—how much is absorbed into blood and how long is it present?

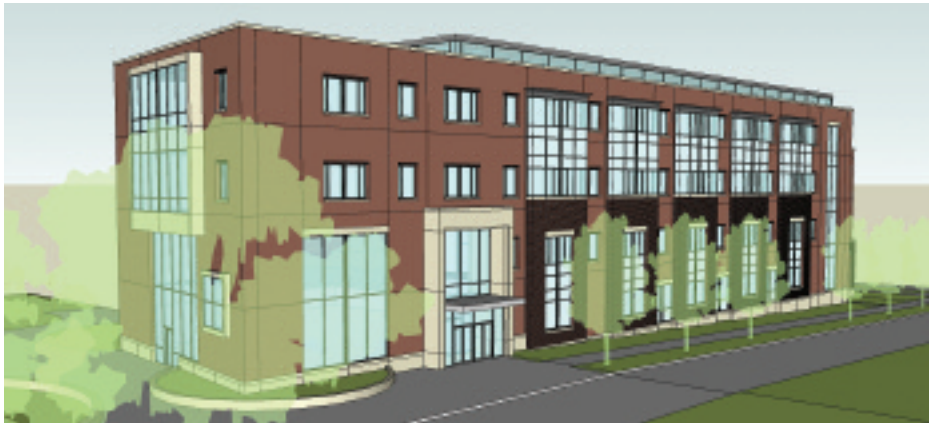
A. About 20-40% of an oral dose is absorbed. It's taken up very rapidly and has a very short half-life in plasma. Within 30 minutes to an hour or so after you take a supplement, maximal blood levels are reached. Within two or three hours it's gone from the blood, either taken up into tissues or excreted.

Q. Does that suggest that lipoic acid is involved in cell-signaling events that continue after the lipoic acid has disappeared?

A. That's correct. It doesn't really accumulate in cells, but it may trigger signaling events that induce some long-lasting effects.

Q. What new programs have been implemented in LPI in recent years?

A. Research in the Institute revolves around the concept of orthomolecular medicine—the right molecule at the right concentration—that Linus Pauling first described in 1968. We are interested in how various micronutrients affect disease initiation and progression, and how they might be used to prevent chronic disease. There are three major disease areas that we are investigating. One is cancer—the Cancer Chemoprotection Program—which is doing very well and is supported by many NIH grants, including a large program project grant that Dr. Rod Dashwood is in the process of renewing, together with Drs. Emily Ho and David Williams. The second group investigates cardiovascular and metabolic diseases. Some exciting new researchers have joined that group, including Dr. Don Jump, who is an expert on fat and carbohydrate metabolism. The third group studies aging and neuro-degenerative diseases, which was recently organized as the Healthy Aging Program. Dr. Tory Hagen, the Burgess and Elizabeth Jamieson Chair in Healthspan Research, is the director of the Healthy Aging Program. The goal of that program is two-fold. First, to better understand underlying mechanisms of aging—what actually changes in our cells and tissues as we age and why energy metabolism and immune function decline. We want to gain a better understanding of normal aging, as well as pathological processes related to aging. The other goal of the Healthy Aging Program is to identify dietary factors and supplements that affect these mechanisms and can be used to extend our healthspan. In other words, how we can stay healthy longer, not just live longer. We have recruited Dr. Fritz Gombart, who is an expert in immune function and immunosenescence, which is the decline of immune function as we age. He has identified some important factors in immune function, especially vitamin D, which acts through an antimicrobial peptide called cathelicidin. Dr. Joe Beckman, the Ava Helen Pauling Chair, leads our research on neurodegenerative diseases, especially ALS. He studies the effect of peroxynitrite on motor neurons and related cells of the nervous system. He is especially interested in how peptides and micronutrients, including zinc and antioxidants, might help protect those cells.



Q. Has the current economic recession affected LPI?

A. Yes, it has. Our endowment, which is a very important source of funding for the Institute, has declined by about one-third. This means we have less money available to support endowed faculty, our outreach and educational programs, and our administrative staff. It's a serious problem, which adds urgency to our current fund-raising efforts.

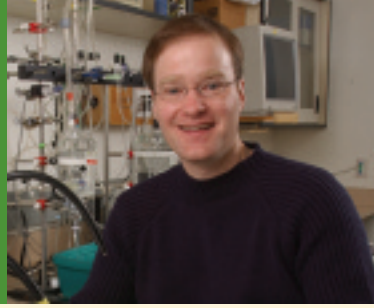
Q. What is your vision for the future of LPI?

A. The future of LPI is tightly linked to our new building, the Linus Pauling Science Center, which will be finished in 2011. The LPSC will house the Linus Pauling Institute and part of OSU's Department of Chemistry in state-of-the-art research and teaching facilities. It will be a working memorial to Linus Pauling, who was an alumnus of Oregon State University. The building will also allow us to expand our research base, enabling additional recruitments into the Healthy Aging Program. Our goal is to have five laboratories in each of the three major areas of research in the Institute: cardiovascular and metabolic diseases, cancer chemoprotection, and healthy aging. We also continue to expand our outreach efforts, including the Micronutrient Information Center, which provides free, scientifically accurate information on vitamins, minerals, phyto-chemicals, and certain foods and beverages. We would like to enhance these efforts to educate people about the important role of diet and lifestyle and supplements in disease prevention, which is becoming increasingly urgent as healthcare costs continue to increase. We plan to get involved in school programs to encourage kids to exercise more and eat healthily, and we are in the process of setting up a study in older adults to investigate the beneficial effects of specific lifestyle changes in maintaining health. We will continue to convene our Diet and Optimum Health conference to communicate our research to our scientific peers and to the public. And, of course, we will continue our education efforts with this newsletter. LPI

The Linus Pauling Institute Healthy Aging Program

Maintaining Health and Vitality in Older Adults through Research and Education on "Age-Essential" Micronutrients

Tory Hagen, Ph.D.
Healthy Aging Program Director
The Burgess and Elizabeth Jamieson Endowed Chair in Healthspan Research



The "Graying" of America: A Longer Lifespan, but Limited "Healthspan"

Average life expectancy is significantly increasing throughout the world. According to current census data, U.S. citizens live approximately 76 years, which represents a 60% increase in mean lifespan in just the last 100 years. Numerous interrelated factors—better pre- and post-natal care, the antibiotic revolution, improved food supply, and early disease detection—have combined to produce this unprecedented surge in longevity.

Even though mean lifespan has increased dramatically, health may not be keeping pace with advances in longevity. According to a recent survey by the U.S. Department of Health and Human Services, 40% of Americans over age 65 exhibit at least one chronic disease, disability, or other functional deficit that limits their normal daily activity. For those who are 85 and older or institutionalized in nursing homes, the figure is over 90%. Therefore, lifespan is currently outpacing "healthspan" —the period of a person's life during which they are generally healthy and free from serious or chronic illness.

Seventy-two million Americans will be 65 years old or older in just 25 years, representing one in five Americans. This "age-wave", along with the elevated risk for chronic diseases and loss of health and vitality that disproportionately afflict the elderly, will dramatically affect American society. For example, 77 million baby-boomers will soon be eligible for social security and Medicare. If tax rates stay at current levels, 76% of all federal income tax revenue will go to fund just these two programs by 2050. The graying of America and increased longevity—but limited healthspan—will dramatically change the country.

Current Gerontological Research is Poorly Focused on Increasing Healthspan

Most of the current effort in geriatric medicine is focused on after-the-fact treatments for specific diseases. Even federally funded research is primarily directed towards disease and not to the basic biological mechanisms that comprise the aging process. For example, over half of the current annual budget of the National Institute on Aging is dedicated to research on a single pathology—Alzheimer's disease.

Developing treatments for age-related diseases is an important goal, but the current approach has yielded only minimal advances —some prolongation of life at the cost of good health. The result is a rampant escalation of U.S. healthcare costs, with 75% of all healthcare dollars currently spent on the elderly. These enormous expenses are destined to rise dramatically in the near future if the current research approach remains the same. The marked increase in the population of older adults and their associated poor health urgently require new healthcare strategies to meet the challenges of a massive increase in the geriatric population.

The Healthy Aging Program of the Linus Pauling Institute: Increasing Healthspan

Recognizing the need for a new approach to maintain health and vitality in the elderly, the Linus Pauling Institute has recently established the Healthy Aging Program. This Program is designed to fill an important gap in gerontological research by targeting the causes that make age the leading risk factor for pathologies like atherosclerosis, cancer, and neurodegenerative diseases. The strategic imperative of LPI's Healthy Aging Program is to move geriatric medicine from reactive and merely palliative care to preventative and protective approaches that prolong health. This health-centered mission is unique among major medical and gerontological programs.

The LPI's Healthy Aging Program emphasizes two areas:

Cellular and molecular "vitality assurance" systems that maintain health with age. Because the basic biology of aging is poorly characterized, there is an urgent need for research that identifies the molecular and cellular mechanisms underlying the aging process. We want to define age-related changes in the body's stress response systems that fail to adequately respond to xenobiotic, oxidative/nitrative, inflammatory, and pathological insults—all identified as major factors that increase risk for chronic disease and morbidity in older adults.

Dietary regimens that limit the risk for developing age-associated diseases. Diet and lifestyle strongly influence the trajectory of overall health throughout life and represent major ways to modify risk for the pathophysiologies of aging. The Healthy Aging Program seeks to define "age-essential" micronutrients and dietary factors that maintain cellular vitality assurance systems, thereby preventing the decline of health. A significant effort will be placed on defining gene-nutrient interactions that affect these systems and determining the optimal intake of phytochemicals and micronutrients, including vitamins, to maintain health.

Establishing the Linus Pauling Institute Healthy Aging Program

Critical research on the role of micronutrients in limiting age-dependent mitochondrial decay—responsible for declines in energy and organ function—and maintaining the regulation of detoxification genes to protect against cell damage and cancer —is already under way in LPI and serves as the foundation of the Healthy Aging Program. However, we recognize that a more comprehensive research focus is needed than what currently exists. To this end, it is envisioned that at least two additional Principal Investigators whose research focus is aligned with the goals of the Healthy Aging Program should be hired to create the critical mass of research needed to reach our long-term goals. Therefore, one of our initial tasks is to recruit outstanding researchers involved in areas such as neurocognitive function, immunosenescence, or epigenetics who also share the goal of defining how dietary factors help maintain these vitality systems into advanced old age. We envision that the existing expertise, new faculty hires and equipment upgrades that will come with the completion of the new Linus Pauling Science Center in 2011 will produce a unique, world-class center for aging research. LPI

Dietary and Lifestyle Strategies to Control Blood Pressure

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

The term *blood pressure* refers to the force of blood exerted against the walls of blood vessels, especially the arteries, as the heart pumps blood to the rest of the body. Blood pressure is expressed in units called "millimeters of mercury" (mm Hg). There are two measurements of arterial pressure: systolic blood pressure and diastolic blood pressure. Systolic blood pressure (SBP), the higher of the two numbers, is the maximum arterial pressure when the heart contracts or beats. Diastolic blood pressure (DBP) is the minimum arterial pressure when the heart relaxes between heartbeats. Blood pressure readings are expressed as systolic pressure over diastolic pressure. For example, a blood pressure measurement of 120/80 mm Hg means that systolic blood pressure is 120 mm Hg and diastolic pressure is 80 mm Hg.

Blood pressure naturally fluctuates throughout the day according to a number of factors, including body temperature, diet, physical activity, emotional state, and use of certain drugs or medications. Normal blood pressure is currently defined as a SBP lower than 120 mm Hg and a DBP lower than 80 mm Hg. Hypotension refers to abnormally low blood pressure, most often defined as a SBP lower than 90 mm Hg or a DBP lower than 60 mm Hg. More of a public health concern, however, is abnormally high blood pressure, called hypertension. Hypertension is defined as a SBP of 140 mm Hg or greater or a DBP of 90 mm Hg or greater (see table below). Prehypertension, defined as a SBP of 120-139 mm Hg or a DBP of 80-89 mm Hg, is not clinical hypertension, but blood pressure readings are elevated above optimal levels. The causes of elevated blood pressure are multifactorial, with genetic and environmental influences. The majority of patients with high blood pressure have decreased elasticity and increased peripheral resistance in blood vessels. Both pre-hypertensive and hypertensive individuals are at a heightened risk for coronary heart disease, stroke, and kidney disease. "White-coat hypertension" is a condition in which a patient displays elevated blood pressure only in a clinical setting, such as a doctor's office, and is probably caused by anxiety.

Categories of Systolic Blood Pressure (SBP) and Diastolic Blood Pressure (DBP) for Adults

Class	SBP (mm Hg)		DBP (mm Hg)
Normal	< 120	and	< 80
Prehypertension	120-139	or	80-89
Hypertension	≥ 140	or	≥ 90

Almost one-third of U.S. adults—72 million Americans—have hypertension, and according to current estimates, nearly as many have prehypertension. Many people do not realize that they have high blood pressure because the disease itself does not usually cause symptoms. Symptoms often manifest only after organ damage, which results from long-term elevation of blood pressure. Thus, blood pressure should be monitored at regular intervals, and high blood pressure should be managed. Although there are a number of pharmaceuticals used to treat high blood pressure, prehypertension and hypertension can often be managed through diet and lifestyle modification, possibly preventing or delaying the need for medication. Such strategies include dietary changes, regular aerobic exercise, smoking cessation, and stress reduction.

A number of dietary changes can help lower blood pressure in people with normal or high blood pressure. High sodium intakes are linked with high blood pressure. Accordingly, several randomized controlled trials have found that dietary sodium or salt reduction lowers blood pressure. One analysis found that modest sodium reduction (1,800 mg/day of sodium or 4.6 grams/day of salt) in individuals with elevated blood pressure lowered SBP by 5 mm Hg and DBP by 2.7 mm Hg. A low sodium diet was also found to reduce SBP by 2 mm Hg and DBP by 1 mm Hg in subjects with normal blood pressure. On average, Americans consume 4,000 mg of sodium (10 grams of salt) daily. Of this amount, about 75% is derived from processed food; only about 5% is discretionary salt use—salt added at the table. Eliminating processed foods with their added salt from the diet dramatically lowers sodium intake and helps control blood pressure. According to USDA recommendations, healthy adults should limit sodium consumption to 2,300 mg daily (5.8 grams of salt), which is about a teaspoon of salt. Individuals who tend to be more sensitive to the effects of sodium on blood pressure —those with diagnosed hypertension, people over 50 years of age, and individuals of African descent—should consume less than 1,500 mg of sodium (3.8 grams of salt) daily.

Compared to a typical American diet, the DASH (Dietary Approaches to Stop Hypertension) eating plan has been shown to significantly lower blood pressure in individuals with hypertension, as well as in those with normal blood pressure. The DASH diet emphasizes fruits, vegetables, whole grains, poultry, fish, nuts, and low-fat dairy products, and compared to the usual American diet, it is markedly higher in potassium and calcium, modestly higher in protein, and lower in total fat, saturated fat, and cholesterol. In the initial DASH trial, sodium levels were kept constant throughout the study in order to better evaluate the effects of other dietary components. The more recent DASH-sodium trial compared the DASH diet with a typical American diet at three levels of salt intake: low (2.9 grams/day), medium (5.8 grams/day, recommended by U.S. dietary guidelines), and high (8.7 grams/day, typical U.S. intake). At each level of salt intake, individuals on the DASH diet had lower SBP and DBP compared to individuals on the typical American diet. This blood pressure reduction was observed in individuals with hypertension and in those with normal blood pressure. The combination of the DASH diet and reduction in salt had an additive effect, lowering blood pressure more than either intervention alone.

Several other dietary factors may affect blood pressure. For instance, consuming excessive amounts of alcohol is associated with hypertension. In fact, drinking more than two alcoholic drinks daily dose-dependently increases blood pressure. Accordingly, heavy drinkers who decrease their consumption of alcoholic beverages experience a

dose-dependent reduction in both SBP and DBP. Moderate amounts of alcohol—two drinks daily for men and one drink daily for women—may not significantly increase blood pressure and, compared to nondrinkers, may actually decrease blood pressure in women. Moreover, moderate consumption of alcohol has been associated with significant reductions in the risk of coronary heart disease, ischemic stroke, and overall mortality. However, drinking more than this amount increases the risk for hypertension, various cancers, and mortality. Thus, limiting alcohol consumption is important for controlling blood pressure. Consumption of caffeine may also affect blood pressure. While caffeine consumption is known to acutely increase blood pressure, considerably less is known regarding the effect of habitual caffeine consumption on blood pressure. Randomized controlled trials examining caffeine or coffee intake on blood pressure have reported conflicting results. One pooled analysis of such trials reported that regular caffeine consumption (as tablets) was associated with a 4.2 mm Hg increase in SBP and a 2.4 mm Hg increase in DBP. However, the blood pressure-raising effect of caffeine was blunted when the caffeine was ingested as coffee, presumably because other compounds in coffee counteract caffeine's effect on blood pressure. Also, several observational studies have found that vitamin C intake or plasma level of vitamin C is inversely associated with blood pressure. Results of intervention trials examining the effect of vitamin C supplementation on blood pressure have been mixed, but most trials have reported beneficial effects with daily doses of 500-1,000 mg. Large-scale, long-term studies are needed to determine whether supplemental vitamin C is effective in preventing or treating hypertension. Additionally, some studies indicate that regular consumption of flavonoid-rich foods, such as wine, tea, and cocoa, may reduce blood pressure. Flavonoids and vitamin C may improve nitric oxide-induced vasodilation, which relaxes or opens blood vessels.

In addition to dietary changes, regular aerobic exercise has been shown to lower resting blood pressure in people with normal or high blood pressure. One pooled analysis of controlled trials found that aerobic exercise significantly reduced blood pressure by an average of 3.8 mm Hg for SBP and 2.6 mm Hg for DBP. The blood pressure-lowering effect observed in this analysis was not dependent on weight loss of participants or on the frequency, intensity, or type of aerobic exercise. Another pooled analysis reported that progressive resistance exercise decreased both resting SBP and DBP by 3 mm Hg. It is not clear how regular aerobic or resistance exercise lowers blood pressure, but suggested mechanisms include decreased peripheral resistance in blood vessels, effects on the nervous and renal systems, and reductions in body weight. Blood pressure reductions resulting from routine physical activity may be more pronounced in overweight or obese individuals compared to those who are normal weight. Individuals should aim for at least 30 minutes of moderate-intensity physical activity, such as brisk walking, most days of the week. Regular exercise is important in the prevention and treatment of hypertension and offers other health benefits as well.

Regular exercise is a key component in weight loss and weight maintenance programs. Weight loss will significantly lower blood pressure in overweight or obese individuals; even a 10-lb (4.5-kg) weight reduction can help control blood pressure. Individuals should aim for a healthy weight with a body mass index (BMI) of 18.5-24.9 kg/m^2. BMI is calculated by dividing weight in kilograms by height in meters squared. Overweight is defined as a BMI of 25.0-29.9 kg/m^2, and obesity is defined as a BMI of 30 kg/m^2 or higher. In addition to having a higher BMI, greater amounts of abdominal fat (waist circumference > 40 inches for men and > 35 inches for women) increase the risk for hypertension. According to the National Heart, Lung, and Blood Institute, overweight and obese individuals should strive for a 10% loss in body weight over a six-month period. Weight loss of 22 lbs (10 kg) generally results in 5-20 mm Hg reductions in SBP.

Smoking cessation and stress relief may also lower blood pressure. It is well-established that cigarette smoking increases the risk of heart disease and stroke in individuals with hypertension. Cigarette smoking causes injury to the vascular endothelium—the single cell layer that lines the inner surface of blood vessels. Thus, cessation of cigarette smoking would decrease one's risk for cardiovascular diseases, in addition to other chronic diseases. Further, stress relief techniques like meditation may help manage blood pressure, although scientific studies are largely lacking.

The interventions discussed above—dietary sodium reduction, adherence to the DASH diet, moderating alcohol consumption, regular exercise, weight loss, and smoking cessation—can help control blood pressure and reduce the risk for cardiovascular diseases. Such modifications may be sufficient to prevent or treat hypertension or may improve antihypertensive drug efficacy in individuals who require medication. These dietary and lifestyle strategies should be discussed with a competent medical professional, who can help personalize a plan to help prevent or treat high blood pressure. Controlling blood pressure will lower the risk of heart disease, stroke, and kidney disease, as well as decrease risk of overall mortality. Thus, reducing blood pressure through changes in diet and lifestyle would result in significant public health benefits.

Useful Web sites:

Dietary sodium and blood pressure:
http://www.nhlbi.nih.gov/health/prof/heart/hbp/salt_upd.htm
http://hyper.ahajournals.org/cgi/content/full/35/4/858

DASH diet:
http://www.nhlbi.nih.gov/health/public/heart/hbp/dash

Weight loss:
http://www.nhlbisupport.com/bmi/bmicalc.htm

Exercise:
http://www.cdc.gov/physicalactivity/downloads/PA_Intensity_table_2_1.pdf

Smoking cessation:
http://www.cdc.gov/tobacco/quit_smoking/index.htm LPI

LPI's Response to the Physicians' Health Study II on Vitamins C and E and the Risk for Heart Disease and Cancer

Two studies published recently in the *Journal of the American Medical Association* (The Physicians' Health Study II) reported that supplemental vitamin C (500 mg/day) or vitamin E (400 IU of synthetic vitamin E every other day) did not reduce the incidence of heart attacks and strokes; deaths from cardiovascular disease; the risk of prostate or total cancers; or cancer mortality in a group of 14,641 U.S. male physicians in a ten-year period.

All of the study subjects were at least 50 years old. Only about 5% had pre-existing heart disease, and about 9% had a history of cancer. They were randomized to receive vitamin C, vitamin E, both vitamin C and vitamin E, or placebo for the duration of the study. The investigators reported good adherence among participants (over 70%, with no differences between groups). There were no significant differences between groups at the beginning of the study—metrics included body mass index, history of cigarette smoking, frequency of exercise, alcohol consumption, aspirin use, and medical history of hypertension, diabetes, high cholesterol, or cardiovascular disease.

There were no statistically significant differences in cardiovascular outcomes, such as heart attacks, heart failure, or angina, between placebo groups and supplement groups, except for the incidence of hemorrhagic strokes. There were 39 such strokes in the vitamin E groups but only 23 in the groups not taking vitamin E, which represents an increased risk of about 75%. Bear in mind that the overall number of hemorrhagic strokes in these groups was small (62 among 14,641 subjects) and eclipsed by the number of ischemic strokes (387). Neither vitamin E nor vitamin C supplements had any significant effect on mortality from cardiovascular disease or total mortality.

The observed increased incidence of hemorrhagic stroke may be related to the known effects of vitamin E on blood clotting: the tolerable upper intake level of vitamin E set by the Food and Nutrition Board is based on its potential impairment of blood clotting. Over 77% of the physicians in the study reported taking aspirin. The combination of aspirin and supplemental vitamin E may have exacerbated the risk for hemorrhagic stroke.

There were no statistically significant differences between the supplement and placebo groups in the incidence of, or mortality from, total cancer or specific cancers, including prostate cancer, colorectal cancer, lung cancer, bladder cancer, pancreatic cancer, lymphoma, leukemia, and melanoma. The investigators noted that several factors, including supplement dose and duration and the good diets of the subjects, may have influenced the outcomes. They did not observe any significant adverse effects of supplemental vitamins C and E. They also noted that their results do not exclude the possibility that supplemental vitamins C and E may have cancer chemoprotective value in the context of other micronutrients, such as a multivitamin supplement.

These results of the Physicians' Health Study II contrast with results from some other large-scale studies. For example, a similar trial in women (The Women's Health Study; 39,900 women at least 45 years old followed for about ten years) who took 600 IU of natural vitamin E every other day for ten years reported a 25% reduction in cardiovascular mortality, as well as a 26% reduction in major cardiovascular events and a 49% reduction in cardiovascular mortality in those women 65 years and older. The Nurses' Health Study (85,000 women followed for 16 years), an observational study (studies that associate diet and supplement intake with disease outcome based on food-frequency questionnaires and other criteria), found that a daily intake of more than 350 mg of vitamin C was associated with over a 25% reduction in the risk for heart disease. Additionally, a meta-analysis of pooled studies (290,000 adults followed for about ten years) reported that the daily intake of at least 700 mg of vitamin C was associated with a 25% decreased risk for heart disease.

Many observational studies have found associations between the intake of vitamin C and reduced cancer incidence, especially of gastrointestinal and lung cancer and, in some subgroups, breast cancer. Generally, daily intakes of less than about 80 to 100 mg of vitamin C were associated with increased risk for cancer. Participants in the Physicians' Health Study II were described as well-nourished, suggesting that their dietary intake of vitamin C may have been sufficient for maximal cancer chemoprotection. Large-scale, long-term observational and randomized controlled studies have not generally found an association between supplemental vitamin E and reduced cancer risk, except for a 15-34% risk reduction for prostate cancer.

The Physicians' Health Study II did not measure blood levels of vitamin C or vitamin E, nor were levels of oxidative stress or C-reactive protein—a marker of inflammation—determined at baseline or at any time during the study. The distinct possibility remains that benefit may have been experienced among those participants with elevated levels of oxidative stress or inflammation at the beginning of the study, assuming that the intake of vitamin C or E was sufficient to attenuate these levels. However, this was not measured in the study and, therefore, is impossible to ascertain. Some studies suggest that the amount of vitamin C for prophylaxis against heart disease may be greater than 500 mg/day and that very high doses of vitamin E are required to significantly attenuate oxidative stress, a presumptive causative factor for heart disease. For example, a Vanderbilt study published in 2007 found that daily doses of 1,600 IU or more of natural vitamin E (*RRR*-alpha-tocopherol) for at least 16 weeks were required to substantially attenuate oxidative stress, as measured by F_2-isoprostanes. Synthetic vitamin E was used in the Physicians' Health Study II, which has only half the bioavailability of natural vitamin E. It is also unknown if the study period of ten years was sufficient to observe long-term effects of supplemental vitamins C and E.

One of the strengths of the Physicians' Health Study II is that it was not observational—specific doses of supplements were taken by the subjects over a long period of time. The studies were carefully executed by skilled investigators and contribute more evidence to the totality of accumulated studies of several different types, many of which have reported benefits for supplemental vitamins C or E in the prevention and treatment of heart disease and cancer. LPI

Developments

Scott Palmer
LPI Director of Development

What Will Your Legacy Be?

Recently a good friend of mine commented that this must be an extraordinarily tough time to be asking people to consider giving to the Linus Pauling Institute. I told him yes; given the huge economic challenges that our nation faces, this is a difficult time for organizations like the Institute that depend so much on the support and generosity of our friends and donors.

The stream of negative financial news and the losses we have all experienced in our retirement and investment accounts fuel growing concerns about the future. People have become much more conservative with their giving. Yet, here I am, asking you and our other friends to consider giving to the Linus Pauling Institute.

The money we get from private gifts has decreased significantly, as has our endowment income. This loss of revenue has had a very real impact on the Institute, and we are looking hard at everything on which we spend money. Deciding what to fund is a difficult task—especially in an organization like the Linus Pauling Institute. But some things are simply more important than others.

Our promise to provide scientifically accurate, evidence-based information and recommendations about micronutrients remains unchanged. We are in an era of instant news where every week a glaring headline about a new study declares that some vitamin or mineral supplement has no health benefit or that a new "miracle" pill will cure whatever ails you. Television is awash in infomercials trying to sell you something to make you healthier.

Every month countless health-oriented reports, articles, and studies are published in dozens of journals and magazines. Just because the results of a new study are trumpeted in the media doesn't mean that it is good science or even accurate. Sifting through this avalanche of information to determine what truly is significant is a laborious and time-consuming task. Just reading studies isn't enough; you have to have trained and knowledgeable people who know what to look for. We do. That's why those who want the latest, most scientifically accurate information about vitamins, minerals, or phytochemicals go to our online Micronutrient Information Center.

We also remain committed to fully funding and developing the Healthy Aging Program. The potential impact of our research on healthy aging is so great that it remains one of our top priorities. Research on aging and neurodegenerative diseases has long been a primary focus of our research.

Last year, thanks to a remarkable gift we received from Burgess and Elizabeth Jamieson, we were able to enhance the scope and scale of our aging research. The Jamieson's gift allowed LPI to establish the Burgess and Elizabeth Jamieson Chair in Healthspan Research. Dr. Tory Hagen was named to hold that chair and was appointed as the Director of our expanded Healthy Aging Program.

Working with his colleagues at the Institute, Tory has created a bold strategic vision for the Healthy Aging Program based on the notion that it is not how long you live that matters, but rather how long you live well. His research agenda for the Healthy Aging Program is as comprehensive as it is innovative. Once fully implemented, it will integrate our existing expertise on the biology of aging with new scientific breakthroughs in epigenetics and immunosenescence and new technologies to create a truly unique team of scientists and researchers with unmatched potential. All it will take is money.

Our goal over the next several years is to raise $5 million to augment the Healthy Aging Program. The money will be used to recruit new scientists, equip labs, launch innovative new research programs, endow new chairs, and create an educational outreach program to make it as easy as possible for our friends and constituents to get information about diet related to aging. Like other key initiatives implemented by the Linus Pauling Institute, the funding for this program will come from your gifts.

The Healthy Aging Program is about the future—your future and mine. Every gift we receive in support of the Program, regardless of size, is important and makes a difference. If you haven't given to the Institute recently, please consider doing so now. If you are interested in supporting this new Program but are unable to make a cash gift at this time, there are a number of planned and deferred giving options available that will enable you to create your own legacy within the Linus Pauling Institute.

For more information about making a cash gift to the Institute or finding out about our planned giving options, including bequests, Charitable Gift Annuities, IRA Gifts and Charitable Remainder Trusts, please feel free to contact me at scott.palmer@oregonstate.edu or (541) 231-6751.

Over the next few months, we will be reviewing and updating our newsletter mailing list. While subscriptions will remain free, the costs of printing and mailing this newsletter are substantial and continue to increase. One way we can save money is to mail the newsletter only to people who want to receive it. If you have any questions about the Newsletter or your subscription, please don't hesitate to contact me. LPI

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

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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

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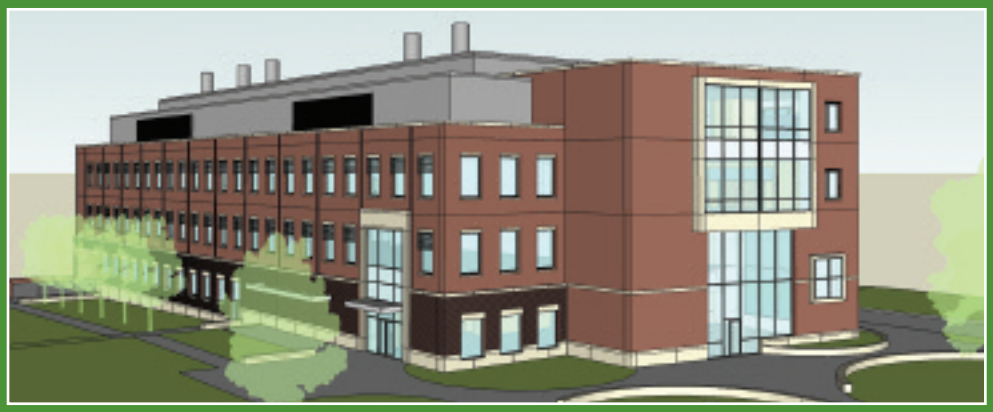

The Linus Pauling Science Center

A rendering of the new Linus Pauling Science Center, scheduled to open in 2011.

Look for these informative articles inside!

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