



OSU Oregon State University

# The Linus Pauling Institute

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



## From the Director

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

It is my pleasure to announce three recent honors awarded to LPI faculty member Dr. Joseph Beckman. Dr. Beckman joined the Linus Pauling Institute in 2001 as Ava Helen Pauling Chair and professor of Biochemistry and Biophysics. Since 2002, he also has served as Director of OSU's Environmental Health Sciences Center, which is funded by the National Institutes of Health and supports research on environmental causes of disease. Last year, Dr. Beckman was bestowed another endowed position in the Institute, the Margaret W. Terrill Research Innovator Faculty Scholar Award, which supports a faculty member of the Linus Pauling Institute "specializing in innovative research projects that are relevant to the mission of the Institute." In addition, in November 2012, the Medical Research Foundation of Oregon Health & Science University presented the Discovery Award to Dr. Beckman "for distinguished achievements in neuroscience." And finally, just this April, Dr. Beckman was awarded the title of Distinguished Professor, the highest honor OSU gives to its faculty.

All of these awards recognize Dr. Beckman's outstanding contributions to our understanding of the causes of amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig's disease. This dreadful disease is caused by the unexplained death of motor neurons that control the movement of all voluntary muscles in our body. We have only about 500,000 motor neurons at birth, and they cannot be replaced once they have been lost. The long-term goals of Dr. Beckman's work are to elucidate the underlying mechanisms of ALS and subsequently identify dietary compounds and design therapeutic drugs that specifically target these mechanisms.

Just a few weeks ago, Dr. Beckman's group published a ground-breaking study in the *Proceedings of the National*

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## Memories

*An Interview with  
Kathy Magnusson, D.V.M., Ph.D.  
Professor of Biomedical Sciences  
LPI Principal Investigator*

**Q. *When did you decide to become a veterinarian?***

**A.** My father was a veterinarian, and two of my brothers became veterinarians, so it's the career that I was most exposed to. I decided early on that would be my career.

**Q. *Where did you grow up?***

**A.** Blooming Prairie, a small town in Minnesota.

**Q. *Did you practice veterinary medicine after earning your degree?***

**A.** Yes. I took care of dairy cows for a couple of years, as well as small animals like pets, but probably 90 percent of the practice involved dairy cows.

**Q. *What motivated you to pursue research?***

**A.** I fell in love with neuroanatomy my freshmen year of veterinary school and was amazed at how much was already known about the brain, but there was also still plenty to be learned. I knew then that I would ultimately go back to graduate school.

**Q. *When you went to graduate school and pursued research, did you continue to practice veterinary medicine or did you give that up in favor of research?***

**A.** I continued to take care of one client. I would go back and do herd health checks for them as a friend while I was in graduate school. What I missed the most from practice was helping the cows to calf. It's a great feeling to help a new life come into the world.

**Q. *Most of your research has focused on the influence of alterations in the N-methyl-D-aspartate (NMDA) receptor on age-related changes in brain functions, such as learning and memory. What are NMDA receptors and how do they work?***

**A.** NMDA receptors are located on neurons in the brain. They use the amino acid glutamate as their transmitter,

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Dr. Joseph Beckman

*Academy of Sciences.* This study showed that oxidative modification—“nitration”—of a single amino acid residue of a specific protein in motor neurons causes these cells to die. Dr. Beckman said, “It was difficult to believe that adding one nitro group to one protein

will make it toxic enough to kill a motor neuron. But nitration of HSP90 (Heat Shock Protein 90) was shown to activate a pro-inflammatory receptor. This begins a dangerous spiral that eventually leads to the death of motor neurons.” Identifying this specific mechanism now opens the door to developing new strategies that prevent nitration of HSP90 and thus could prevent motor neuron death and ALS. Through the common link of inflammation, Dr. Beckman’s discovery may also relate to other health problems, such as heart disease, chronic pain, and spinal cord injury.

Another LPI faculty also recently received a prestigious award. Dr. Maret Traber, the Helen P. Rumbel Professor for Micronutrient Research in the Institute and professor in OSU’s School of Biological & Population Health Sciences, was bestowed the 2013 Pfizer Consumer Healthcare Nutritional Sciences Award by the American Society for Nutrition. According to ASN, this award is given for contributions “of contemporary significance to the understanding of human nutrition.”



Dr. Maret Traber

Dr. Traber’s pioneering research on vitamin E caused a paradigm shift in our understanding of the mechanisms regulating vitamin E bioavailability and metabolism in humans. Much of her work has focused on a protein in the liver, the alpha-tocopherol transfer protein, which plays a critical role in

maintaining adequate vitamin E levels in the body. Using zebrafish as an experimental model, Dr. Traber recently found that this protein is also necessary in the developing brain, likely to deliver vitamin E as the brain is forming. This discovery may have important implications for determining human vitamin E requirements during conception and early pregnancy.

Dr. Traber has served on the National Academy of Science’s Institute of Medicine Panel on Dietary Antioxidants and Related Compounds, which established the most recent recommended dietary allowances and other dietary reference intakes for vitamins C and E, selenium, and carotenoids.



Jana Zvibleman

Finally, I’m delighted to introduce our first Communications Officer in the Linus Pauling Institute. This position was inspired and funded by one of our most loyal and generous supporters, whose crucial gift will help LPI communicate our research discoveries to enable everyone to achieve optimum health. Our new colleague Jana Zvibleman is a long-time editor and writer who served as the Public Relations Officer in OSU’s Research Office for the past 14 years. I’m already enjoying how her creative ideas and unique perspectives are helping us refresh and expand our outreach and communications efforts. I’m sure you will be interested in her new LPI blog and other strategies that she is developing to keep you informed about the activities of our researchers and how their work can make a difference in your life. If we don’t yet have your email address and you would like to get occasional, timely communications, please send it to Jana at [jana.zvibleman@oregonstate.edu](mailto:jana.zvibleman@oregonstate.edu).

Thank you, and enjoy the Newsletter! **LPI**

## Does Carnitine Cause Heart Disease? — Balz Frei, Ph.D.

You may have heard or read about carnitine—found in red meat or taken as a supplement—as a possible risk factor for heart disease. Many of you may be taking dietary supplements containing carnitine, often in combination with lipoic acid. This combination, as well as carnitine taken alone, is believed to increase fat burning and improve energy metabolism, especially in older adults.

According to the new study, a metabolite of carnitine called trimethylamine oxide (TMAO) is formed by gut bacteria (intestinal microbiota) and absorbed into the body. High plasma levels of carnitine in humans were found to be associated with an increased risk of cardiovascular disease, including heart attack and stroke. Furthermore, in mice TMAO enhanced cholesterol absorption and altered cholesterol metabolism in the liver and in monocyte-macrophages (inflammatory white

blood cells), thereby promoting atherosclerosis (the underlying cause of cardiovascular disease).

My immediate reaction to the study is that it is intriguing and novel but far from definitive. I certainly wouldn’t draw any conclusions at this point with respect to heart disease risk in humans. The possible culprit, TMAO, comes also from many other sources besides carnitine, such as choline, choline phospholipids, acetyl choline, and even fish, which we know lowers cardiovascular risk. In fact, a 1999 study found that eating 8 oz. of marine fish, such as cod, haddock, halibut, or herring, elevated urinary levels of TMAO and its precursor, trimethylamine, by about 35 times, whereas 8 oz. of beef, pork, or other meats did not. Moreover, while carnitine levels were associated with an increased risk of cardiovascular disease in the study, TMAO itself was not.

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but glutamate binding itself is not sufficient to open up an NMDA receptor channel to make it work—you have to have the neuron already excited or depolarized. And typically that involves other glutamate receptors. Once that occurs—the glutamate is present and the cell has been excited—then the NMDA receptor channel can open up and function to create memories. The reason it doesn't open without those conditions is that there is a magnesium block. That's believed to be important for memory because you need a stronger stimulus than just normal signaling to activate the receptor. That way you're not making memories all the time; you're making memories when it's important—there's enough of a signal.

**Q. When you say the glutamate receptor opens up, what does that mean?**

**A.** There are four protein subunits that make up the receptor. When the glutamate binds to the receptor, there's a change in the relationship of those subunits, such that a channel is formed. Then, when the cell is depolarized, the magnesium block, which is within the channel, is let loose. Then the channel can conduct calcium ions through the NMDA receptor channel into the neuron. The calcium is the key to making changes within the neurons that lead to memory formation.

**Q. And without that activity, no memory is formed?**

**A.** The NMDA receptor is important in a lot of different brain regions, but there are some brain regions that don't require the NMDA receptor for memory formation. There are other receptors involved in making memories, but this is probably the most important.

**Q. Are NMDA receptors concentrated in certain areas of the brain, and if so, why?**

**A.** They are concentrated in the higher centers of the brain—the cerebral cortex and the hippocampus—because those are the regions where memories are made and stored.

**Q. Are the different types of memory affected by NMDA receptor activity?**

**A.** Well, we know that long-term memory, which is also called reference memory, is influenced by NMDA receptor activity. But we also see that short-term memory is affected, too. If you block NMDA receptor activity in the frontal cortex during and for ten minutes after a learning experience, the memories are not retained. For shorter periods of time, the NMDA receptor doesn't appear to be involved.

**Q. Are there other types of cognitive activity affected by this?**

**A.** Flexibility is affected. There's evidence that if you block the NMDA receptors in the prefrontal cortex people can't adjust to new rules.

**Q. How do you study these phenomena?**

**A.** I examine the NMDA receptor by receptor-binding autoradiography on tissue sections. This involves labeling glutamate with radioactive isotopes and having it bind to the cellular receptors so they can be visualized.

**Q. Are those tissue sections from rodents?**

**A.** Those are mainly from rodents. Amazingly, you can take a frozen brain section, thaw it, and you can still get glutamate to bind to these receptors to then look at the density changes that occur with aging.

**Q. How do you correlate that to specific memory function in rodents?**

**A.** In the rodents, I've done memory testing using the Morris water maze for spatial long-term memory, and then I correlate that with the receptor-binding changes we observe in tissue sections.

**Q. How does the Morris water maze work?**

**A.** It's basically a horse water tank, and we color the water to make it opaque. We make it white because we use black mice and that makes a nice contrast for our tracking system. There is a platform hidden just below the surface when we're doing long-term memory testing. There are visual cues on the walls of the room and high up on the tank—there's evidence from other studies that rodents use those cues like we would use landmarks. So we put the rodents into the tank at different entry points, and they swim around for a maximum of one minute or until they find the platform. It acts as a resting place for them. If they're going to get better at finding that hidden platform, they've got to use the cues around the room, make a spatial map, and use it again. I've heard it said that mice aren't natural swimmers like rats, but we actually have to train them to stay on the platform, which gives them time to make their spatial map. Without that training, some of them would just keep swimming.

**Q. Does NMDA receptor activity affect the performance assessed by the Morris water maze test?**

**A.** I have not done the specific functional studies that showed that; Morris and others have done electrophysiology and have shown correlations between changes in the electrophysiology and performance in the Morris water maze. They have also blocked the function of the NMDA receptor with drugs and shown deficits in the water maze test. What I've done is to look at the expression of the different subunits that make up the receptor. Changes in expression in some of them correlate with the long-term and delayed short-term memory declines with age, as assessed in the Morris water maze test, and some of them don't.

**Q. Do NMDA receptors function about the same in rodents and people?**

**A.** As far we know, they function the same.

**Q. Are rodents good models to study the molecular basis of memory function?**

**A.** Yes, I think so. There's little difference in the NMDA receptor between rats and humans. Drugs like PCP or ketamine that block NMDA receptors in humans have similar effects in rodents. We wanted to look at the age-related changes in the GluN2B subunit in humans to see

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if it was similar to our findings in the mice. Human brain banks have normal brain tissue from older individuals available, but it's more difficult to find enough normal young brain tissue, probably because young adults don't have the same motivation to set up a donation plan. So, rodents remain the best model.

**Q. Why are the NMDA receptors more vulnerable to dysfunction in older age?**

**A.** That is something I haven't figured out yet. I think that one of the subunits, the GluN2B subunit, undergoes a programmed decline, meaning that the expression of the gene has been shut down or reduced. This subunit is very important during development of the brain and has very high expression at birth. The expression of the GluN2B subunit declines across post-birth development, and we think that this continues during aging. The GluN1 subunit seems to be more affected by the animal's environment or experience. The effects of aging are more variable on the GluN1 subunit.

**Q. If that particular gene—GluN2B—becomes dysfunctional as animals age, does that mean that the subunit is not being made correctly and that the overall receptor is not functioning properly?**

**A.** Exactly, it's not as good at making a memory because when the GluN2B subunit is present, the receptor and channel will stay open longer and have a higher affinity for glutamate. When we increase the gene expression in the brain by introducing more copies via a virus, memory is improved. But we have to be careful not to overdo it—too much NMDA receptor activity can be

damaging. Our goal is to restore memory similar to that in young adults, not try to super-size it.

**Q. The NMDA receptor binds glutamate, which is a non-essential amino acid in proteins that we consume in our diet, and glycine, which is sweet-tasting and the smallest amino acid. Does the binding efficiency of both amino acids decline with age?**

**A.** No. In our studies, the glycine-binding site does not appear to change significantly with age, but the glutamate site does.

**Q. Does supplementing with glutamate have any affect on NMDA receptor activity?**

**A.** That's not known yet. There are issues with excitotoxicity if you raise glutamate levels excessively in the brain.

**Q. Does the number of NMDA receptors decline with age as well?**

**A.** Yes. Studies have shown that there's a decline in the absolute number, especially in certain regions of the brain. However, there also appear to be changes in the function of the remaining receptors.

**Q. Does oxidation play a role in age-related changes in NMDA function?**

**A.** Tom Foster's work with electrophysiology has shown there is a redox site on the NMDA receptor that is in a more oxidized state in an old rat.

**Q. Does that suggest that antioxidants like vitamin C or vitamin E, which is fat-soluble, might have an effect?**

**A.** Vitamin C does not appear to reverse the oxidized state

### Continued from page 2 — Does Carnitine Cause Heart Disease?

Only after some statistical manipulations did the authors find an association between increased heart disease risk and increased levels of carnitine and TMAO combined.

It is possible that carnitine and TMAO levels in humans are only markers of meat intake rather than causative agents in heart disease. Just because carnitine levels are associated with increased heart disease risk in humans does not mean carnitine (or TMAO) *causes* heart disease; correlation is not necessarily causation. Much more work needs to be done to establish causality in humans. To call carnitine a culprit in heart disease (as the press labeled it) is premature. The main culprit in red meat is saturated fat, and I don't think carnitine or TMAO will get even close to saturated fat in terms of causing atherosclerosis and increasing cardiovascular risk in humans. Relative risk is important, and we know the major coronary risk factors in humans.

There is a large body of work accumulated over decades showing that acetyl-L-carnitine and lipoic acid are safe to use at appropriate doses. Acetyl-L-carnitine

alone has been given clinically at very high doses (as much as 8 grams per day) with no observed side effects, including those suggested by this study. We know from rodent studies that acetyl-L-carnitine improves mitochondrial function, lowers lipid levels (which would lower cardiovascular risk), and improves muscle function and cognition. Carnitine has health benefits, and its "benefit-to-risk" ratio needs to be taken into account. This new study is the only one suggesting that carnitine is associated with atherosclerosis. I believe that, on balance, current evidence favors the use of carnitine supplements. However, we must prudently and continually reassess our recommendations based on new scientific information.

For now, I continue to take my combined lipoic acid and carnitine supplement and will also continue to monitor my heart disease risk—which is very low due to a highly favorable lipid profile (low total and LDL cholesterol, high HDL cholesterol, low triglycerides), normal blood pressure, normal blood glucose, healthy body weight and waist circumference, and low inflammation (plasma CRP levels). In addition, I exercise regularly and avoid red meat. **LPI**



of the NMDA receptor when directly applied to brain slices from aged rats. I don't believe it has been assessed via treatment of the whole animal. Vitamin E given in the diet can increase the density of NMDA receptor binding across all ages of animals. Although this doesn't appear to counteract the effects of aging directly, an increase in receptors could still help to restore learning ability.

**Q. *Is inflammation implicated in the age-related decline of NMDA function?***

**A.** Yes. A study by Mesches and coworkers showed that giving the anti-inflammatory drug sulindac caused an increase in the GluN1 and the GluN2B subunits and improvement in working memory in aged rats.

**Q. *Do dietary constituents like omega-3 fatty acids, carnitine, lipoic acid, and blueberry extracts improve NMDA function in old animals?***

**A.** Those have been shown in a number of studies by other researchers to increase expression and/or function of the NMDA receptor.

**Q. *Does dietary restriction affect NMDA receptor activity?***

**A.** We've shown that the binding density of the NMDA receptor is improved by dietary restriction, especially involving the GluN1 subunit.

**Q. *Do you have any plans to work with LPI Principal Investigator Viviana Perez to see if the dietary restriction mimetic rapamycin might have a similar effect?***

**A.** Our Healthy Aging Program group has regular meetings to find ways to collaborate, and I think that would be a perfect project. Rapamycin has been shown to enhance memory in aged animals. It would be interesting to know whether this was through an action on NMDA receptors.

**Q. *Aside from glutamate-binding efficiency, are there other differences in NMDA function between young and old animals?***

**A.** Others have shown that long-term potentiation is impaired in old animals, and that affects memory. This is observed in hippocampal brain slices. To study this, you do an electrical stimulation in the slices once every 15 seconds to get a baseline response, which is based on the opening up of the non-NMDA glutamate receptors and ions flowing away from the recording electrode. If you then do a high-frequency stimulation—the original one was about 100 Hertz—and then go back to the lower frequency every 15 seconds, there's a bigger response. And that response in live animals has been shown to last from weeks to months. That's referred to

as long-term potentiation. You can induce long-term potentiation in slices from old animals, but it decays faster than it does in a young animal—it's not maintained. The NMDA receptor is important for the initiation of long-term potentiation in many brain regions.

**Q. *Does stress or exercise affect NMDA receptor activity and memory function?***

**A.** I believe that they do. We have evidence that behavioral experience, such as the Morris water maze test, can increase one of the GluN1 splice variants, and this increase is associated with improved NMDA receptor function. At this point we don't know whether it's the learning or the exercise that's influential.

**Q. *When you were at Colorado State University you assessed the effect of certain metals like lead, magnesium, and zinc on the NMDA receptor and memory function in rats. What did you learn?***

**A.** I learned that we were using way too high concentrations that were non-dietary and non-physiological. I was working with a toxicologist, and he was interested in very high concentrations that you might find in a toxic superfund site. There's a zinc-binding site on the receptor. Magnesium is important for the blockade of the receptor, and there's evidence that lead can influence NMDA receptors. So we thought that it would be good to look at a mixture.

**Q. *With other LPI investigators you've looked into the effect of age on dendritic cells and immune function in mice. What are dendritic cells and what role do they play in immune function?***

**A.** Dendritic cells are cells that are in your skin, blood, liver, and lungs. They monitor the body for invaders. They're job is surveillance, and if they detect a foreign substance they'll engulf it and go to the nearest lymph node to recruit lymphocytes. In collaboration with Emily Ho and Carmen Wong, we found age-related declines in some types of dendritic cells in the spleen and increases in others. In addition, we found that dendritic cells in aged animals did not respond as robustly to challenge as those from young.

**Q. *In collaboration with LPI's Emily Ho and her team, you also investigated the effect of zinc deficiency on immune response and inflammation. What did you find in those studies?***

**A.** We found that decreased zinc within immune cells was associated with age-related increases in inflammation. The decreased zinc appeared to be due to alterations in zinc transporter expression. Dietary supplementation with zinc was able to reduce inflammation in the aged animals.

**Q. *You participated in another study that assessed the effect of zinc supplementation on immune function in old mice. Why does good zinc status improve immune function in those animals?***

**A.** Zinc supplementation was able to increase the number of thymocytes. This was associated with increased

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maturation of thymocytes and inhibition of a thymic suppressive cytokine, which is a cellular signaling molecule. Increased thymocytes should enhance immune responsiveness in aged individuals.

**Q. Where is your research headed?**

**A.** As far as the NMDA receptor is concerned, we're still trying to characterize what's gone wrong with it with age. We've also begun to try interventions to see if we can repair or prevent the damage.

**Q. Using dietary micronutrients?**

**A.** We're interested in looking at alpha-lipoic acid and carnitine with LPI's Tory Hagen because his group has shown that those substances improve memory in rats, so that seems like an obvious thing to try to see if they have effects on the NMDA receptor. We see different changes with age in different regions of the brain. And alpha-lipoic acid and carnitine have an effect on mitochondria, so one of my ideas is that there may be a differential amount of mitochondrial damage in

different brain regions. The health of the mitochondria in those areas might affect the oxidative environment, and we already know that the receptor is in a more oxidized state in older animals.

**Q. Do you have more collaborations planned with other LPI scientists?**

**A.** Yes. We're currently collaborating with Fritz Gombart, looking at vitamin D and vitamin D deficiency, which is prevalent in a lot of older individuals. We are also beginning a collaboration with Fred Stevens to see whether the anti-inflammatory or fatty acid-reducing effects of xanthohumol may help NMDA receptor expression and/or memory.

**Q. Finally, what do you like to do when you're not in the lab?**

**A.** I like to walk my dogs and watch them run.

**Q. What kind of dogs do you have?**

**A.** Two Springer Spaniels, Lily and Divya.



## Anti-infective Vitamins

Stephen Lawson  
LPI Administrative Officer

**D**r. Fritz Gombart of LPI has studied some surprising attributes of vitamin D for many years. The “sunshine vitamin” is not actually a vitamin because it is synthesized in the skin upon exposure to sunlight. It's been known since the early 1920s that vitamin D deficiency in a child's developing years causes rickets, a severe skeletal deformity. Vitamin D regulates calcium, which is crucial in bone formation. Many other functions of vitamin D have been discovered in recent years, including a role in cell differentiation, leading to the normal maturation of cells. Vitamin D deficiency has been implicated not only in rickets but also in osteomalacia (bone pain) and muscle weakness and pain. Additionally, vitamin D may be useful in blood pressure regulation and preventing cardiovascular disease, osteoporosis, and cancers of the colon, breast, and prostate.

Vitamin D plays a critical role in immunity by regulating T-lymphocyte production. In association with colleagues at Cedars-Sinai Medical Center, Dr. Gombart found that vitamin D regulates the production of an antimicrobial peptide in immune cells—neutrophils and macrophages—called cathelicidin, which can kill bacteria and viruses. This observation may explain why tuberculosis patients benefited from exposure to sunlight and is being followed up with studies on dialysis patients vulnerable to infections and increased mortality. Most recently, Dr. Gombart and colleagues found that blood levels of vitamin D up to

32 ng/mL (80 nmol/L)—a level many experts believe to be sufficient—were correlated with increasing levels of cathelicidin in blood.

In the new study, published in *The Journal of Clinical Investigation* in September, 2012, Dr. Gombart and his colleagues at Cedars-Sinai reported that another vitamin—niacin, or vitamin B<sub>3</sub>—also has potent immune effects. Severe niacin deficiency results in pellagra, which causes a skin rash, dementia, and death. Pellagra was common in the southeastern United States in the early 20<sup>th</sup> century when corn was widely consumed as a major dietary staple that had not been soaked in lime to release niacin and increase its bioavailability.

In the new study, researchers found that injecting nicotinamide—a form of niacin—into mice infected with *Staphylococcus aureus* dramatically cleared the infection, acting by increasing the activity of neutrophils through the stimulation of a transcription factor that regulates the production of antimicrobial peptides, including cathelicidin. Similar results were obtained with human blood taken from healthy volunteers and pretreated with nicotinamide before being exposed to *S. aureus*. These experiments demonstrate that nicotinamide may have value in preventing bacterial infections. There is also some evidence that nicotinamide may be useful in treating tuberculosis and HIV infection, as well as for killing other pathogens like *K. pneumoniae* and *P. aeruginosa*, bacteria responsible for pneumonia and sepsis, respectively. High-dose, supranutritional nicotinamide used in these experiments can cause unpleasant side effects and is not recommended for common use. More research is necessary to find out what dose or dietary intake of niacin in humans may be prophylactic. **LPI**

**Q. Is there an advantage in having a veterinary school and hospital here at Oregon State University, in terms of community access to good veterinary services?**

**A.** Oh yes, I think so. I think we've got excellent basic health care with the practitioners in this area, but OSU can offer more specialized services that the average practitioner wouldn't normally do on a daily basis.

**Q. Do you think we might commonly have health insurance for pets some day?**

**A.** I don't know, but the attitude towards pets has really changed over the last 20 years, and it could be possible in the future. More and more people, myself included, consider their pets as part of their family. At the veterinary school, there have been situations in which accommodations were made for a student whose pet was seriously ill, similar to if it had been a child. I don't think a veterinary school would have done that 20 years ago. So those attitudes are changing. **LPI**



## Multivitamin/mineral Supplements and Cancer

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

**M**ultivitamin/mineral supplements (MVMs) are popular dietary supplements taken by about one-third of Americans. It is not yet known whether taking a MVM might prevent chronic or age-related disease; previous findings of observational studies and randomized controlled trials (RCTs) on MVM supplementation and disease have been mixed. Results from a new study—the largest and longest RCT to date—indicate that MVMs offer older, well-nourished men some protection against cancer, suggesting that a healthy diet alone may not be sufficient to meet micronutrient needs for health promotion in this population.

The new Harvard study, the Physicians' Health Study II (PHS II), examined the effect of MVMs on the primary prevention of cancer, cardiovascular disease, age-related eye diseases, and early cognitive decline. In this trial, 14,641 male physicians (50 years or older at the beginning of the study; mean age of 64.3 years) were randomized to receive either a daily MVM or placebo; the men did not know which supplement they received. Men with a history of cancer, heart attack, or stroke at the time of study enrollment were not excluded from the study. Participants were followed for an average of 11.2 years.

Most notably of the PHS II results, MVM supplementation lowered the incidence of, that is, reduced the risk for overall cancer by 8% (total cancers excluding nonmelanoma skin cancers); this effect was stronger in men aged 70 years or older and in men with no parental history of cancer. Additionally, MVMs reduced overall cancer occurrence in men who had a personal history of cancer at the time the study began (1,312 of the study participants). Statistical power was limited to detect an effect of MVMs on the incidence of individual cancers, but excluding cases of prostate cancer from total cancers resulted in a significant, 12% reduction in cancer risk. Furthermore, PHS II found a nonstatistically significant trend for a 12% decreased risk for cancer mortality. MVM use in the PHS II, however, did not reduce risk for cardiovascular disease or total mortality in older men.

Thus, the major finding of PHS II is that MVM supplementation reduced risk of total cancers. While an 8% reduction in risk is modest, such a decrease would have public health significance, possibly preventing up to 130,000 cases of cancer each year in the U.S. alone. It is important to note that the PHS II trial was conducted in a group of well-nourished, older physicians with a lower than average fraction of current smokers (<4% of study participants). Therefore, MVMs appear to offer added benefit in a healthy population of older men. It is possible that some groups of people, such as those with high-stress jobs, have higher requirements for vitamins and nutritionally essential minerals, and a healthy diet alone may be insufficient for health promotion in such populations.

Previous research on MVM supplementation and chronic disease, mainly from observational studies, has been inconsistent. The PHS II trial is the largest and longest double-blind, placebo-controlled trial of MVM supplementation to date. While both observational studies and RCTs have inherent limitations, RCTs are considered the "gold standard" in medicine and are the only type of study that can establish causation; observational studies can only show associations that may or may not be causally linked. One of the largest observational studies looking at MVM supplementation, the Women's Health Initiative in 2009 that followed 93,676 women for 7.9 years, found little difference in total mortality or in the incidence of cancer or cardiovascular disease between women regularly taking MVMs and those who did not. A large-scale RCT in women is needed to determine whether long-term adherence to a MVM might have benefits similar to those reported for men.

In summary, the PHS II found a modest but significant cancer preventive effect of MVMs in older men, especially the elderly, and noted a trend for decreased cancer-related mortality in men taking the MVMs. Few adverse side effects were reported, and the safety of MVMs that provide the daily values (DV) of micronutrients (i.e., not megadoses) is generally well recognized. It's important to note that the MVM used in PHS contained 25 micronutrients, most at levels at or below the DV. The benefits observed in this study might be larger in a less well-nourished population suffering from micronutrient inadequacies. While such dietary supplements appear to offer added benefit, a healthy diet, maintaining a healthy weight, regular exercise, and avoiding tobacco remain critically important for the prevention of cancer and other chronic diseases. **LPI**



## What is Metabolism?

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

The word “metabolism” commonly appears in nutrition news, but what does it actually mean? The term encompasses so many concepts that entire textbooks are written about it, while research laboratories devote their full effort to studying just one aspect of metabolism.

### Definitions

The dictionary defines metabolism as the sum of the processes in the build-up and destruction of protoplasm. There are three key concepts included in this definition:

- *Protoplasm* is the mixture of organic and inorganic substances that constitute the living cell.
- *Anabolism* is the build-up of protoplasm. It involves a series of chemical reactions to construct molecules from smaller units.
- *Catabolism* is the destruction of protoplasm. It involves a series of chemical reactions that break down molecules into smaller units.

Also important in this definition is the *cell*, the basic unit of life. Our bodies are a mixture of cells and proteins organized into complex structures like tissues and organs. Meanwhile, food is a complex mixture of atoms (the basic unit of matter) and molecules. So when we eat, we are providing both our bodies and our cells the materials necessary to support metabolism.

### Energy Metabolism

You may hear people bemoaning their “slow metabolism” and how it foils their attempts to lose weight. In this context, one is referring to energy metabolism—all of the biochemical processes that convert food into useable energy and material. Energy metabolism can be further described at the whole-body and cellular levels.

### Whole-body Metabolism

Whole-body metabolism refers to the sum of all metabolic reactions in the body and is represented by a very simple equation known as the energy balance equation:

$$\text{Energy intake} = \text{Energy expenditure}$$

Energy intake comes from the calories in the food we eat; specifically, from the calories in the macronutrients: carbohydrate provides 4 kilocalories (kcal) per gram (g), fat provides 9 kcal/g, and protein provides 4 kcal/g. Note that in nutrition, calorie and kilocalorie are used interchangeably to denote the energy content of food. Technically, a calorie is the amount of energy required to raise the temperature of one gram of water by one degree Celsius.

The expenditure side of the equation is comprised of three variables: basal metabolic rate (BMR), physical activity, and the thermic effect of food (TEF).

BMR represents the energy expended to maintain fundamental functions of the body. BMR is the largest contributor to total energy expenditure with 60-75% of our total energy output fueling basic activities of life, such as respiration, circulation, body temperature regulation, and synthesis of new cells and tissues. BMR itself is influenced by several factors, including body composition, age, gender, and genetics.

- *Body composition.* Lean body mass (LBM) is the primary determinant of BMR. The more lean tissue (muscle tissue without fat) you have, the higher your BMR.
- *Age.* There is a 3-5% decrease in BMR per decade of life after age 30. This is largely attributed to the loss of LBM that occurs with age.
- *Gender.* BMR is higher in men than women.
- *Genetics.* Inherited differences in genes (e.g., the FTO gene variant linked to obesity) and hormones (e.g., thyroid hormone) that participate in the regulation of metabolic pathways can influence BMR.

Physical activity accounts for 15-35% of total energy expenditure. Physical activity encompasses all types of muscle movement; thus, dedicated exercise sessions as well as daily activities like walking constitute physical activity. Not only does physical activity itself expend energy, but it can also increase LBM and, consequently, one’s BMR.

TEF represents the energy expended to digest, absorb, transport, metabolize, and store food. Five to ten percent of total energy expenditure goes to TEF. Some diet fads will encourage consumption of certain types of “calorie-burning” foods because it is energetically costly to process them with slightly higher TEF values. The contribution of an individual food to total energy expenditure is very small; it is much more effective to increase physical activity and decrease calorie intake for weight loss (for example, a 500 kcal/day imbalance will result in a weight loss of one pound per week) and to reap a myriad of health benefits.

**Metabolic disorders.** Metabolic syndrome is a composite of metabolic disturbances that increase one’s risk for developing cardiovascular disease and type 2 diabetes. The risk for developing metabolic syndrome itself is closely linked to obesity and lack of physical activity, both of which contribute to a chronic imbalance in whole-body energy metabolism. The pathophysiological causes and effects of metabolic syndrome are not yet fully known. It appears that abdominal adiposity and insulin resistance are the most critical risk factors leading to its development. Inflammation may also be an important contributor. Please visit the Disease Index in LPI’s online Micronutrient Information Center (MIC) and the Cardiovascular and Metabolic Diseases (CMD) research group (<http://lpi.oregonstate.edu/>) for more information on metabolic syndrome.



## Cellular Metabolism

During digestion, food is broken down into its constituent molecules, which are absorbed into the blood stream and then presented to the cell. Carbohydrates consist of molecules of glucose and fructose, dietary fat (primarily in the form of triglycerides) contains three fatty acid molecules attached to a glycerol molecule, and proteins are built of linked amino acid molecules.

Once inside the cell, energy metabolism relies on an organelle known as the mitochondrion (see figure below). The mitochondria are often referred to as the “power plants” of the cell because they convert the chemical energy in food to a form of usable energy known as adenosine triphosphate (ATP). ATP must be constantly generated in order to fuel all of the fundamental processes that comprise BMR.

## Macronutrient and Micronutrient Metabolism

Nutrients are the chemicals in food that are essential to human growth and function. They are generally divided into two major classes: the macronutrients and the micronutrients. In terms of energy metabolism, the macronutrients provide chemical energy, while the micronutrients function as essential cofactors, or assistants, required for obtaining the energy provided by macronutrients. Micronutrients are so named because they are required only in very small amounts compared to macronutrients.

## Macronutrient Metabolism

The macronutrients provide energy and the building blocks of large biological molecules like proteins and membrane lipids. A mixture of carbohydrates (starches and sugars) and fats are oxidized to maintain a constant supply of ATP to support daily activities and exercise. The proportion of each substrate used varies with the type and duration of physical activity. Carbohydrate is especially important for ATP production during exercise, while much of the energy used during rest comes from fat. Though dietary protein provides calories, carbohydrate and fat are the preferred substrates for energy metabolism, sparing protein for other vital bodily functions. The body can use protein as an energy source during illness, trauma, and when stores of carbohydrate and fat are low, but this limits the availability of protein for essential basal metabolic requirements.

## Micronutrient Metabolism

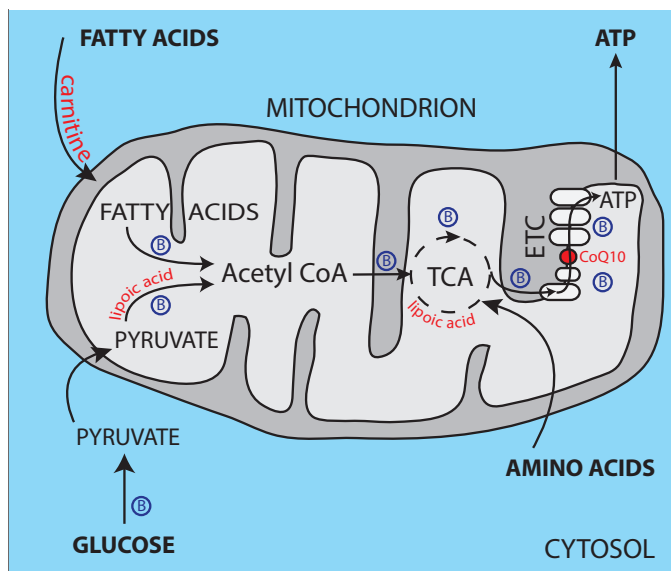
Micronutrients do not directly provide energy; rather, they assist in the acquisition of energy provided by the macronutrients.

**B vitamins.** The B vitamins in particular serve an important role as cofactors in energy metabolism. Energy metabolism consists of a series of enzymatic reactions that convert the molecules in food to ATP. Enzymes do not work in isolation—they require several vitamins to help them carry out the metabolic reactions. Each of the B vitamins (thiamin, riboflavin, niacin, pantothenic acid, vitamin B<sub>6</sub>, biotin, folate, and vitamin B<sub>12</sub>) serves as a cofactor in various aspects of carbohydrate, fat, and protein metabolism.

Because the enzymatic reaction requires B-vitamin cofactors, deficiency in one or more B vitamins could restrict the rate of the reaction, becoming a “rate-limiting” factor. The Dietary Reference Intakes (DRIs) are intake recommendations for healthy individuals, designed to prevent deficiency disease and to reduce the risk of chronic disease when sufficient scientific evidence exists. The recommended intakes for the B vitamins are set at levels to maintain sufficient plasma concentrations and to ensure optimal activity of the enzymatic reactions in which they participate.

**Lipoic acid, L-carnitine, and coenzyme Q<sub>10</sub>.** In addition to the B vitamins, several other nutrients operate in energy metabolism. Lipoic acid, L-carnitine, and coenzyme Q<sub>10</sub> are conditionally essential nutrients that can be synthesized inside our bodies and obtained from foods and dietary supplements. Each participates in different aspects of cellular energy metabolism inside mitochondria (see figure below).

- **Lipoic acid.** Alpha-lipoic acid is derived from a short-chain fatty acid precursor and contains two thiol (sulfur-containing) groups, important for the functional activity of lipoic acid. Lipoic acid is incorporated into the structure of several enzymes involved in cellular energy metabolism and serves as a critical functional group in the oxidation of macronutrient-derived substrates. While protein-bound lipoic acid functions in energy metabolism, free lipoic acid can function as an antioxidant, a metal-chelator, and in glucose uptake and utilization.



**Figure legend.** Mitochondria convert the chemical energy contained in food molecules (fatty acids, glucose, amino acids) into a form of cellular energy known as adenosine triphosphate (ATP). Sites of lipoic acid, coenzyme Q<sub>10</sub> (CoQ<sub>10</sub>), and carnitine involvement are highlighted in red. The B vitamins (Ⓟ) operate at sites all along the entire path of macronutrient oxidation (black arrows). A single scheme is depicted, but in actuality, these steps are occurring at multiple sites throughout the mitochondrion at all times. Additionally, the number of mitochondria present in a single cell can range from approximately 200 to 2,000 depending on the cell type. Abbreviations: ETC, electron transport chain; TCA, tricarboxylic acid cycle.

*continued on page 10*



## Better Dairy Products with Omega-3 Fatty Acids

Gerd Bobe, Ph.D., M.P.H.  
Assistant Professor of Animal Sciences  
LPI Principal Investigator

Health and nutrition professionals advise people to limit consumption of saturated fatty acids and to increase the consumption of foods rich in omega-3 and other polyunsaturated fatty acids, which have anti-inflammatory properties and may help prevent cardiovascular disease. Traditional cattle feed contains a mixture of corn, grains, alfalfa hay, and grass silage that results in milk and dairy products with low concentrations of omega-3 and other polyunsaturated fatty acids. Adding flaxseed, a rich source of omega-3 fatty acids, to cows' diet may improve the nutritional value of milk and dairy products. To determine the amount of flaxseed that would maximize the amount of omega-3 fatty acids in milk and dairy products without negatively affecting their texture and production, cows were fed up to six pounds per day of extruded flaxseed—flaxseed that was ground and then pressed into pellets with heat. Milk from these cows was then turned into butter and fresh cheese, which were tested for texture and nutrient composition. Feeding up to six pounds per day of extruded flaxseed to cows improved the fatty acid profile without negatively affecting the production and texture of the milk, butter, and cheese. Saturated fatty acids in whole-milk fat from supplemented cows decreased 18%, polyunsaturated fatty acids increased 82%, and omega-3 concentrations increased 70% compared to milk from cows that were not fed flaxseed. Similar improvements were observed in butter and fresh cheese. In addition, refrigerated butter was softer and less adhesive, thanks to fewer saturated fatty acids. **LPI**



### VISIT OUR NEW LPI BLOG

<http://www.blogs.oregonstate.edu/linuspaulinginstitute>

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

Alice A. Winans  
Harold H. Plaut  
Helen M. Watkins  
Nancy J. Bradford

### Continued from page 9 — What is Metabolism?

• *L-carnitine*. Carnitine, chemically derived from the amino acids lysine and methionine, is necessary for the transport of long-chain fatty acids into the mitochondria. In healthy people, carnitine balance is maintained through endogenous biosynthesis of L-carnitine, absorption from dietary sources, and elimination and reabsorption by the kidneys. Nutritional carnitine deficiency is very rare, but the potential benefits of carnitine supplementation in humans is an active area of research.

• *Coenzyme Q<sub>10</sub>*. Coenzyme Q<sub>10</sub> is a fat-soluble compound and integral component of the electron transport chain (ETC) inside mitochondria. The ETC is a series of steps by which electrons are transferred between complexes embedded within the inner matrix of mitochondria. It is via the ETC that our cells couple the oxidation of the molecules in food to the generation of energy as ATP.

Dr. Tory Hagen, the director of LPI's Healthy Aging Program, has termed lipoic acid and acetyl-L-carnitine "age-essential" micronutrients and is investigating their ability to counteract mitochondrial dysfunction that occurs as we age. Acetyl-L-carnitine has an added acetyl group and is better absorbed and better distributed in the body than L-carnitine. For more information about lipoic acid, acetyl-L-carnitine, and coenzyme Q<sub>10</sub>, please visit the "Other Nutrients" section in our Micronutrient Information Center (MIC).

### Other Types of Metabolism

*Xenobiotic metabolism*. While energy metabolism involves the conversion of dietary molecules to usable cellular energy, xenobiotic metabolism involves the conversion of foreign molecules to manageable forms. More specifically, xenobiotic metabolism involves a series of enzymatic reactions that convert a foreign chemical compound (the prefix "xeno" means stranger) into an inert substance that can be safely excreted from the body. It can be divided into three phases. In phase I, also referred to as activation, oxygen is used to form a reactive site on the xenobiotic compound; members of the cytochrome P450 (CYP) family of enzymes participate in phase I metabolism. Phase II, or conjugation, involves the addition of a water-soluble chemical group to the reactive site of the phase I metabolite. And finally, in phase III, the solubilized compound is expelled from the cell and then excreted from the body.

Drugs, toxins, steroid hormones, and carcinogens are substrates for enzymes involved in xenobiotic metabolism. One of the ways certain phytochemicals (non-nutrient plant chemicals found in fruits and vegetables) may benefit health is by inducing or inhibiting enzymes involved in xenobiotic metabolism. For example, animal studies demonstrate that metabolites of indole-3-carbinol (I3C), a compound present in cruciferous vegetables, can induce the expression of various phase I and phase II enzymes, potentially enhancing the detoxification and elimination of carcinogens.

By this same mechanism, certain phytochemicals may interact with prescribed medications that share xenobiotic (or biotransformation) enzymes, potentially altering the effective dose of the drug. As always, it is important to inform your healthcare provider of any dietary supplements you are taking.

**First-pass metabolism.** First-pass metabolism is a phenomenon of drug metabolism whereby the concentration of a drug is greatly reduced before it reaches the systemic circulation due to action in the gastrointestinal tract and liver. The amounts of drugs prescribed account for first-pass effects so that a sufficient amount of active agent will reach the target tissue. Phytochemicals are also subjected to first-pass metabolism. As a result, the form of the phytochemical in circulation is very different from that of the ingested compound. Therefore, it's very important to recognize that the health effects of many phytochemicals are likely due to their metabolites and not to the parent compound found in food.

## Conclusion

Stated very simply, metabolism is a biochemical conversion. We convert food to ATP (energy metabolism), we convert medications to inert compounds (xenobiotic metabolism), and we convert plant chemicals to various bioactive forms (first-pass metabolism). Each metabolic reaction requires the coordinated activity of several proteins, enzymes, and cofactors, all of which rely on proper and adequate nutrient intake for optimal function.

This article has touched on only a few basic concepts of metabolism and is by no means comprehensive. Please see the MIC for more detailed information on each of the micronutrients mentioned above. **LPI**

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## Developments

Michele Erickson  
LPI Director of Development

The Linus Pauling Institute has made remarkable strides in recent years. 2013 marked the 17<sup>th</sup> year of LPI's move from Palo Alto, California, to Oregon State University. We have achieved many of our benchmarks in those years, including the two most recent ones:

- The Healthy Aging Program has fulfilled our vision to have five pre-eminent Principal Investigators working together to elevate the Institute to become a globally recognized authority in healthspan research.
- LPI's investigators are now under one roof in our new, state-of-the-art research facility—the Linus Pauling Science Center.

To capitalize on this momentum, we turn now to setting the course for LPI's next 15 years. At this pivotal moment, input from our closest friends, including you, is vital. We would be pleased to have you help us shape the vision for the Institute's future.

The pull-out response card in this newsletter provides two new opportunities for you to participate in this process. First, in an effort to expand our communication with you, we would like to include you in the group of our supporters who receive occasional email messages from Director Balz Frei and others at the Linus Pauling Institute about our work. We are also launching an LPI "blog" to bring you insights about our research discoveries, personalized messages from our principal investigators, and another way for you to offer your thoughts. Sharing your email address with us will help make you an insider with the Linus Pauling Institute.

A second option on the response card invites you to support the Institute's future with a planned gift. LPI's Legacy Circle members are important participants in shaping a stable and sustainable future for the Linus Pauling Institute.

All of us at the Linus Pauling Institute appreciate your dedication to our mission to conduct research and provide education to help people everywhere achieve healthy, productive lives and to prevent disease through nutrition, dietary supplements, and lifestyle. We hope you will enhance your relationship with us by returning the response card in this newsletter, enabling us to communicate in new and meaningful ways. **LPI**

Michele Erickson  
Director of Development  
Linus Pauling Institute

# 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  
Web site: <http://lpi.oregonstate.edu>



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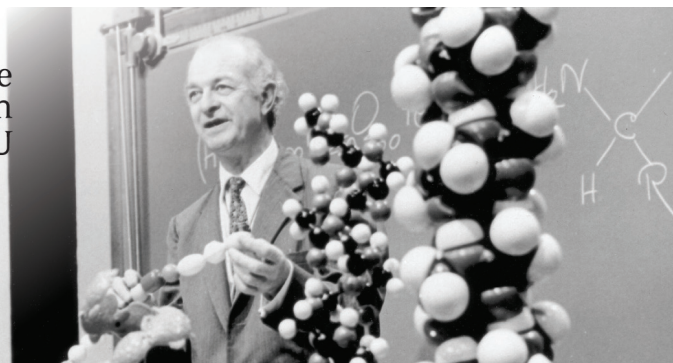
*Special thanks to Barbara McVicar for editorial assistance and photographs, authors of signed articles, and Dick Willoughby for the logo photograph of Linus Pauling.*



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