KLRI

Gray is the new Gold

STATE OF THE SCIENCE
TWO THOUSAND EIGHT

LONGEVITY SCIENCE:
SLOWING THE SPIRAL OF AGING

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Kronos Longevity Research Institute

State of the Science: 2008

It might seem that an organization with the word "longevity" in its name would be dedicated to discovering ways to lengthen the human lifespan. But that's not the only focus of the Kronos Longevity Research Institute (KLRI), or, in fact, of most of the world's leading research institutions that concentrate on aging issues. Instead, the goal is to find a way to make the final third of our lives as rich and full as the first two-thirds by helping us maintain our health and independence.

Doing that requires teasing out markers of aging beyond chronology, wrinkles and gray hair. What provides the best clues to an individual's "real" age? Is it loss of muscle mass? Insulin resistance? Changing hormone levels? And then, once you identify those clues, what are the underlying biological systems responsible for them?

Only through this process of scientific discovery can the real contributors to aging and age-related diseases be identified and, when possible, reversed.

In this State of the Science Report, we provide an update on three important areas of research at KLRI and with scientists connected with KLRI: oxidative stress, hormonal changes, and nutrition and its effects on diabetes. All are separate and yet, as you will see, interrelated, bound by their influences on biochemical factors such as inflammation, insulin resistance, and DNA abnormalities that underlie much of human disease these days. ◊

EXECUTIVE Summary

Take away the chronological age, wrinkles and gray hair and what do you have left when it comes to evaluating someone's true age? Quite a lot, as it turns out. From measuring levels of oxidative stress and hormones to defining healthy aging through diet, researchers from the Kronos Longevity Research Institute (KLRI) and their scientific peers have been moving beyond the obvious to identify underlying markers of aging and develop ways to slow their effects.

Only through this process of scientific discovery can the real contributors to aging and age-related diseases be identified and, when possible, reversed.

This State of the Science report provides information on several key areas of aging-related research involving KLRI scientists and their peers around the country. Specifically:

Oxidative stress. KLRI researchers, often in conjunction with scientists at academic centers throughout the country, are trying to learn how oxidative damage occurs, how it can be prevented or minimized, and how aging affects innate mechanisms designed to protect against it. To that end, KLRI scientists have developed methods to accurately measure oxidative stress biomarkers in the urine and blood. Now, using a unique process that safely creates oxidative stress in healthy humans, they are working to establish a reference range for people ages 20 to 85 to determine what is "normal" in different age groups. This will help identify age-related differences in oxidation and validate the existence of oxidative stress biomarkers in age-related diseases.

Caloric restriction. A major initiative in anti-aging research focuses on the effects of caloric restriction on longevity and age-related disease. Animal studies find that dramatically reducing food intake can significantly increase longevity. Now researchers affiliated with KLRI are trying to identify compounds that produce the same biochemical effects without limiting the amount of food—research that could revolutionize the way we age.

Hormones. Along with oxidation and caloric restriction, the impact of hormones is a key focus in the area of longevity research, particularly at KLRI. Here, researchers have launched a national, multicenter study to evaluate the effects of estrogen therapy in women just before and after menopause—something the highly publicized Women's Health Initiative (WHI) tried to do. Because WHI participants were much older than women who typically use postmenopausal hormone therapy, its results have become suspect. The KLRI study is designed to evaluate the effects of supplemental estrogen on markers of cardiovascular health as well as cognition and memory in a "real world" environment with younger women.

Researchers from KLRI have also launched a large, national study to evaluate the risks and benefits of testosterone therapy in older men. The TEAAM study will enable researchers to see if supplemental testosterone increases the risk of atherosclerosis and evaluate its impact on lean body mass levels, muscle development, cognitive function, and health-related quality of life.

KLRI researchers have also developed a unique study to evaluate the effects of increased levels of human growth hormone—without supplementing with the hormone—on markers of aging.

Nutrition. Finally, KLRI researchers are beginning to tease out the impact of diet on aging-related conditions such as insulin resistance, which is thought to contribute to a variety of diseases common in older people. The first study evaluated the anti-inflammatory effects of high levels of omega-3 fatty acids, "healthy" fats found primarily in fatty fish like salmon and tuna and in some seeds.

Through these and other studies, researchers at KLRI and around the world are working to create a new understanding of aging and a new paradigm of "old age." Their goal is to insure that regardless of how long one lives, every day, every year, is lived as fully as possible, with the best possible quality of life and health. ◊

OXIDATIVE STRESS: A Key to Aging

Ever left your kid's wagon outside for a couple of weeks only to find the once-bright red paint covered in rust? Tossed a few cut-up apples into a salad then found them soft and brown an hour later when you're ready to serve? If so, then you've experienced the same biochemical reaction that is slowly wearing down your cells, damaging your tissues, and potentially shortening your life: oxidation.

Every time you breathe, metabolize food, or exercise, every time your cells create energy to keep you going, damaging byproducts called oxygen-free radicals are also created. Think of them as a cellular form of exhaust, similar to the exhaust your car creates as a byproduct of the gasoline it uses for energy. Only instead of polluting the air, this form of biochemical "exhaust" can damage nearby tissues, leading to age-related diseases like cancer, heart disease, diabetes, osteoarthritis, and Alzheimer's disease. In essence, the very processes that promote life inevitably lead to death.

Thankfully, your body has mechanisms in place to protect against such damage called, appropriately enough, antioxidants. They include enzymes like glutathione, proteins like albumin, chemical compounds like uric acid, and vitamins like E and C. Their sole function is to mop up free radicals and render them harmless or, at the very least, weaker. But over time, the thinking goes, this antioxidant defense system becomes weaker.

This so-called "free radical theory of aging" is just one of several hypotheses about the causes of aging, but it's one with 30 years of research behind it and a growing cadre of believers. It is also one that KLRI and Kronos Scientific Laboratory (KSL) have chosen as part of their research focus.

While oxidative stress is not the only mechanism behind aging, says KLRI Director and President, S. Mitchell Harman, MD, PhD, "the damage by oxygen-free radicals to tissue components *is* a very important contributor to the aging process." Particularly, he notes, if you define aging (as many do) as a "progressive disorganization of cells and tissues with a resulting loss of adaptive ability to cope with the environment."

Translation: The older we get, the more damage we're likely to incur from daily living. For not only do the power plants of cells, the mitochondria, produce more free radicals, it becomes harder for our innate system to deactivate them. The result? Damaged DNA, RNA, lipids, and proteins.

Thus, KLRI researchers, often in conjunction with scientists at academic centers throughout the country, are trying to learn more about how that damage occurs, the processes designed to prevent or minimize such damage, and the effects of aging on those protective mechanisms. Such knowledge, says Dr. Harman, will provide a critical piece of the aging puzzle. ◊

Think Twice About Taking Supplemental Antioxidants

Do you take a daily multivitamin? Chew some vitamin C when you feel a cold coming on? Swallow an antioxidant combo to protect your eyes? If so, you're not alone. Millions of Americans take nutritional supplements, many because they believe the extra antioxidants helps prevent disease and damage.

However, KLRI Director and President, S. Mitchell Harman, MD, PhD, notes that, "there is no scientific data at all" that supplementing with such antioxidants prevents oxidative damage and reduces disease risk in humans. In fact, several studies found that such supplementation could actually *increase* oxidative damage and disease.

The best example of this occurred in two National Cancer Institute studies designed to evaluate how large doses of antioxidants affected the risk of lung and other cancers in current and former smokers. In the first, called the Alpha-Tocopherol Beta-Carotene Cancer Prevention Study (ATBC), 29,133 Finnish male smokers received high doses of beta carotene, vitamin E, both, or a placebo for five to eight years. The results shocked researchers: Those taking beta carotene had 18 percent more lung cancer and eight percent more deaths than those not taking it.[1]

A similar study called CARET (Beta Carotene and Retinol Efficacy Trial) evaluated a combination of beta carotene and vitamin A on more than

Think Twice *continued*

18,000 former and current smokers, as well as men exposed to asbestos (another cause of lung cancer). It found similar results. After an average of four years, those taking supplements had 28 percent more lung cancers and 17 percent more deaths.[2]

The problem is that giving people supplemental antioxidants disrupts the delicate balance between too much and too little that your body always tries to maintain, called homeostasis. It works like this: When free-radical activity increases, signals go out to increase production of antioxidants. Once the free-radical activity is under control, those antioxidant levels return to "normal." But if those signals of increased free-radical activity never get sent, your innate antioxidant systems never "learn" to produce the appropriate levels of protection.

"The theory is that certain free radicals are functioning as messengers or key molecules to which the body's internal antioxidant system responds," explains Christopher B. Heward, PhD, president of Kronos Science Laboratory (KSL). But if you supplement with antioxidants and keep free-radical levels abnormally low, those key molecules never appear, and your own internal system of creating antioxidants fails. Then if the supplemental antioxidants are strong enough to control free-radical production, oxidative stress and tissue damage occurs.

"That's why it's so important the research be done on these antioxidants," notes Dr. Heward. ◊

DEFINING the Damage

It's pretty easy to test theories in the laboratory. You want to measure oxidative damage on cells? Pull out the cells' DNA and expose it to high concentrations of oxygen and metal ions and see how it goes. You want to see how well an antioxidant sops up oxygen-free radicals? Add some vitamin E or C and watch. When it comes to measuring oxidative damage in humans, however, the picture is much more complex and challenging. Until recently, scientists used a variety of markers to estimate oxidative stress and its damage, but there was no standard technique.

"People in this field had done a lot of elegant chemistry to understand the chemistry of free-radical reactions and oxidative stress, but the methods available to translate that into what goes on *in vivo* (in humans or animals) were terrible," said L. Jackson "Jack" Roberts II, MD, professor of pharmacology and medicine at Vanderbilt University Medical Center, and a member of KLRI's Scientific Advisory Board. "It was a real impediment to the entire free-radical field because we couldn't prove what we saw in the laboratory in living models."

To that end, KLRI and KSL researchers have spent years developing a set of assays, or tests, to accurately measure oxidative stress biomarkers in the urine and blood. These include measures of RNA, DNA, lipid, and protein damage. In fact, KLRI enhanced an assay that was developed by Christiaan Leeuwenburgh, PhD, chief of the department of aging and geriatric research at the University of Florida, to assess of oxidative damage in key proteins by measuring dityrosine, a molecule produced only as a result of free-radical damage and, working together with Dr. Roberts, to measure isoprostanes, prostaglandin-like compounds created by free radicals.

"With this battery of damage markers, we feel like we're in a great position to evaluate individuals in populations who may be under greater-than-normal oxidative stress," said Dr. Heward. It also makes KSL the only laboratory in the world that can simultaneously measure markers for the four primary forms of free-radical damage.

This is important because different types of oxidative damage occur in different people at different times. Measuring just one component of damage, as most laboratories do, is like measuring the weight but not the height of an individual when determining if that person is overweight. Evaluating damage to the various cellular components simultaneously, however, provides a fairly complete assessment of an individual's oxidative stress status.

KLRI scientists are now working to establish a reference range for people ages 20 to 85 to be able to determine what is "normal" in different age groups. Then they can see if there are age-related differences in oxidation and validate the biomarkers with age-related diseases. ◊

Cigarette Smoking and Oxidative Stress

To validate their oxidative stress assay panel, KLRI researchers tested it in cigarette smokers, comparing levels of oxidative stress markers in smokers to those in ex-smokers and nonsmokers. Why cigarette smoking? Because it is a highly oxidative process.

Everyone knows that people who smoke are more likely to develop lung cancer and heart disease, and to have high blood pressure and high cholesterol, among other medical conditions. But just how cigarette smoking contributes to these conditions is still unclear. One likely mechanism is oxidation. Many chemicals within cigarettes contribute to a significantly increased production of oxygen-free radicals. Plus, because smoking creates such high levels of oxidative damage, it rapidly uses up antioxidants. So, for instance, in your lungs such high levels of free radicals easily overwhelm any protection offered by your own internal defense system. This, in turn, leads to significant damage of the cells' DNA and the production of abnormal cells. Over time, these abnormal cells can become cancers.

Smoking also oxidizes LDL cholesterol, setting into motion a chain of events that ends with lesions in artery walls, the earliest sign of atherosclerosis. The oxidative stress from smoking also interferes with enzymes that help arteries expand and contract, leading to high blood pressure and damaging artery walls, contributing further to atherosclerosis.

Instead of just studying this oxidative damage in the laboratory, today KLRI scientists can actually measure it in smokers, providing even stronger evidence of the damage the habit wreaks. ◊

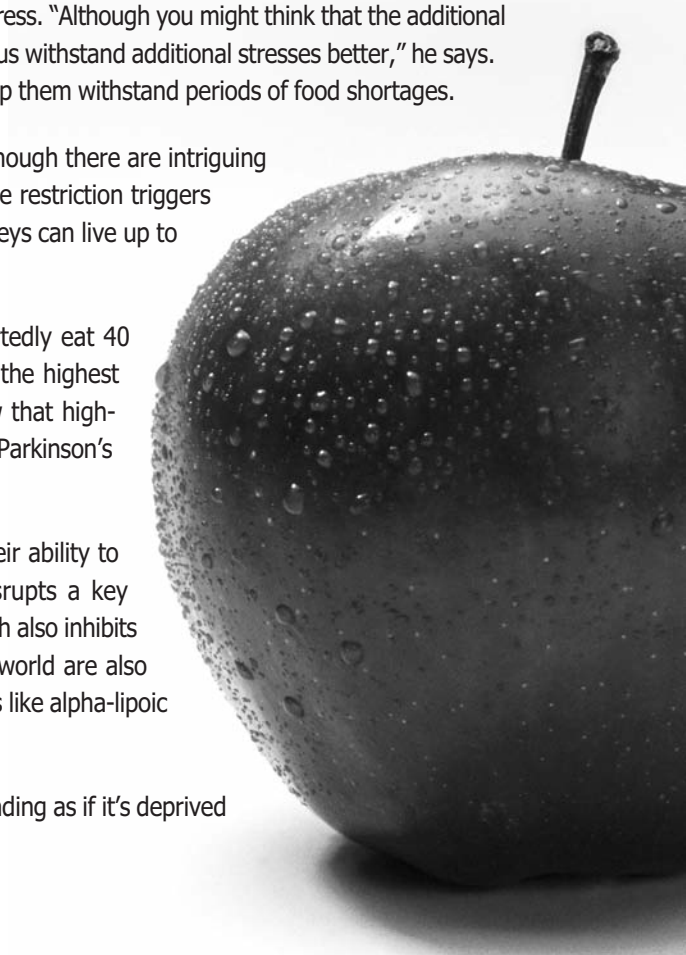
SLOW the Damage

It sounds simple on the surface: Eat less and you'll live longer. At least, that's what studies in animals ranging from fruit flies, worms, and rats to dogs and primates find: Cutting calories by one-third to one-half significantly increases life spans and reduces disease.[3,4] Since few people want to trim their calories by a third (what would life be without molten chocolate cake and ice cream?), KLRI Scientific Advisor Donald K. Ingram, PhD, and his team are searching for compounds that mimic the biochemical effects of eating less, "fooling" cells into behaving as if they were deprived of energy.

Doing this, studies show, slows metabolic processes, in turn triggering a biochemical cascade that reduces heart rate and body temperature, slashes insulin production, and helps maintain higher levels of "anti-aging" hormones like DHEA that tend to drop as we age. It also contributes to lower levels of cholesterol and triglycerides and more-normal blood-glucose levels, all of which, in turn, can reduce the oxidation and inflammation that contribute to numerous medical conditions, including heart disease and diabetes.

Just what's going on? No one is quite sure yet, says Dr. Ingram, who is professor of the Nutritional Neuroscience and Aging Laboratory at the Pennington Biomedical Research Center at Louisiana State University in Baton Rouge, but there are several theories. One is that the less glucose, or fuel, from food that enters cells, the more efficient mitochondria have to be in creating energy. Thus, just as a catalytic converter reduces the amount of exhaust produced by a car's engine, resulting in less pollution, the mitochondria produce fewer free radicals, resulting in less oxidative stress.

At the same time, he says, it's likely that the stress of calorie restriction redirects energy away from normal growth and reproduction into mechanisms designed to protect the organism from that stress. "Although you might think that the additional stress would be harmful, it actually improves our stress response and helps us withstand additional stresses better," he says. All organisms have these mechanisms built into their cellular memory to help them withstand periods of food shortages.



But will it work in humans? We really don't know yet, says Dr. Ingram, although there are intriguing clues that it will. Studies in rhesus monkeys, for instance, show that calorie restriction triggers the same biological changes as in other animal models. Since rhesus monkeys can live up to 40 years, however, we don't know yet if they'll also live longer.

But consider that people living on the Japanese island of Okinawa reportedly eat 40 percent fewer calories than those living on the mainland; they also have the highest percentage of people 100 and older in the country. Conversely, we know that high-calorie diets and obesity lead to increased rates of cancer, Alzheimer's, and Parkinson's disease, as well as heart disease.

To date, Dr. Ingram and others have tested numerous compounds for their ability to mimic calorie restriction, including 2-deoxy-D-glucose (2DG), which disrupts a key enzyme involved in the cellular processing of glucose, and iodoacetate, which also inhibits cellular metabolism, albeit differently from 2DG. Researchers around the world are also looking at things like resveratrol, found in grapes and red wine, antioxidants like alpha-lipoic acid, and even certain medications currently used to treat type 2 diabetes.

"The goal is to find a safe compound that will trick the organism into responding as if it's deprived of energy," Dr. Ingram says. ◊

Oxidative Stress in the Older Adults

"One thing that bugged me when I was a resident in internal medicine was seeing old people come into the hospital with pneumonia and then their kidney function would fail, all their other systems would fail, and they would die," recalls Dr. Roberts. "I always wondered, 'Why is that?' And the answer always was, 'Because they're old.'" But Dr. Roberts thought there had to be a better reason why a reasonably healthy 80-year-old would come into the hospital with a very treatable disease like pneumonia and then die. After years of studying free radicals and oxidative stress, he hypothesized that perhaps older people lose their capacity to adapt to oxidative stress as they age, so a stressful event like pneumonia, in which free-radical production is substantially increased, simply overwhelms their innate antioxidant response resulting in significant tissue damage and death.

Testing that theory, however, proved challenging, because if you want to measure oxidative stress in a human, or test the effects of an antioxidant on oxygen-free radicals, you have to subject that human to a stressful situation designed to increase normal free-radical production. Obesity, diabetes, cigarette smoking, even a stroke, or heart attack would do it, but, obviously, you can't induce such conditions in study participants. That's when Dr. Roberts and his colleagues devised a unique model called forearm ischemia/reperfusion.

The procedure uses an inflated blood pressure cuff to reduce the blood supply to part of the arm for 10 minutes. When the cuff is deflated, highly oxygenated blood rushes back into the arm, triggering a short-term burst of oxygen-free radicals. Do this three times, with a two-minute break in between each cuff inflation, assess the blood of volunteers for markers of oxidative stress, and voila! a perfect model for measuring oxidative stress in humans without creating long-term harm. It's also a perfect model for measuring the effects of diet, lifestyle, supplements, and medications on that stress, and to compare individual responses to oxidative stress as they age.

While Dr. Roberts and his Vanderbilt colleagues had no problem finding young people on whom to test the model, they couldn't find enough healthy older people for their studies. Enter KLRI. Almost overnight, KLRI researchers, including Dr. Harman and Tinna Traustadóttir, PhD, associate director of exercise sciences, recruited 16 healthy, non-smoking men and women 60 or older to test the blood pressure cuff model.

They exposed the older volunteers and a similar group of volunteers in their 20s and 30s to the reperfusion test, then measured levels of isoprostanes in their blood, a marker for oxidative stress, as well as production of antioxidants. While both groups showed significant oxidative stress and antioxidant production, within four hours isoprostane levels in younger participants returned to normal, whereas levels were still 20 percent higher than normal in older individuals, suggesting a reduced resistance to oxidative stress in older people. "They can't seem to adapt to the oxidative insult," explain Dr. Roberts.

"This is what we think happens with aging," he says. "Every time there's any kind of increase in oxidative stress your ability to counteract it and return to baseline gets weaker. Over time, this creates permanent damage in the cell's processes."

But exactly why does this occur? "What's the defect?" Dr. Roberts muses. "Aging causes a defect, but what is it and can we fix it?"

The answer may be found in *C elegans* worms, an animal model often used in genetic research. Preliminary studies find that exposing young worms to oxidative stress "turns on" various genes that help them adapt to later exposure, but this same genetic process does not occur in older worms. Now Dr. Roberts and his team are trying to knock out these genes in young worms to see if they can turn young worms into older worms, then try to "fix" whatever goes wrong with the genetic process in older worms. "If we find the same problem with older people that we find with worms, then maybe down the road in 10 to 20 years we could give people a treatment that would stimulate the same genetic response seen in younger people," he says. "That would make a huge impact in geriatric medicine." ◊

Of Hormones and Aging

Oxidative stress is, of course, just one player in the orchestra of aging. Another is declining hormone levels.

Any woman past 50 knows the impact of this sign of aging: dry skin, loss of libido, vaginal changes, lack of energy, hot flashes. But what she may not know is that the loss of her major reproductive hormone—estrogen—may also dramatically increase her risk of heart disease, osteoporosis, and dementia. It makes sense, then, to assume that replacing that lost estrogen after menopause might, in fact, protect her against heart disease—which is just what numerous observational studies have found over the past 30 years.[5]

So you can understand why, when the first major study to examine this hypothesis in a major randomized clinical trial showed just the opposite, researchers around the world were stunned. The results from the Women's Health Initiative (WHI), reported in the summer of 2002, described a 24 percent increased relative risk of cardiovascular disease in women taking the hormone therapy Prempro, a combination of conjugated equine estrogen (CEE) and a progesterone called medroxyprogesterone acetate (MPA).[6]

Dr. Harman was one of those shocked by the results. "Many of us who had been doing work in the field for years felt that there was something wrong here," he recalls. "The results just didn't make sense."

What leapt out at him and others was the very design of the WHI. Rather than evaluating the impact of hormone therapy on women soon after menopause—which is typically when women start taking supplemental estrogen[7] —the average age of women in the WHI was 63. Most of the women were at least 10 years past menopause when they first started taking estrogen.

"We already knew from the Heart and Estrogen/progestin Replacement Study (HERS) that there was no benefit to taking estrogen if you had preexisting coronary artery disease," Dr. Harman says. "And by the age of 63, how many of them had plaque?"

Indeed, when investigators crunched the numbers again, they found a *reduced* risk of cardiovascular disease and stroke in women who were less than 10 years out from menopause and taking supplemental estrogen.[8]

By the time that analysis was published in 2007, however, Dr. Harman and KLRI had already launched the Kronos Early Estrogen Prevention Study (KEEPS). The study is designed to provide prospective data on the risks and benefits of hormone therapy in *recently menopausal* women, particularly as it relates to the progression of atherosclerosis. "We felt the American public had been done a disservice in the way (the WHI) data had originally been interpreted and we needed to do a study to reexamine this issue," explains Dr. Harman.

Throughout the KEEPS study, researchers will track progression of carotid intimal medial thickness and the build-up of coronary calcium, both markers of atherosclerosis. The data will also be analyzed for any effects of estrogen on women's cognition, including memory and learning ability.

When the study ends sometime around 2011, women should finally have the answers they need about the effects of hormone therapy on cardiovascular health after menopause. ◊

TESTOSTERONE: Estrogen for Men?

For all the jokes about "man-opause," the reality is that a 60-year-old man has significantly lower levels of testosterone, his primary reproductive hormone, than a 30-year-old. While men don't experience the sudden drop in testosterone as women do with estrogen, the decline is real. They lose about 1 to 2 percent a year until, by the time they reach 80, an estimated 70 percent have levels low enough to be considered problematic.[16] And the problems are numerous. Low testosterone levels are linked with low libido, muscle mass, bone loss, type 2 diabetes, metabolic syndrome, heart disease, and Alzheimer disease.[17,18,19,20,21]

Which begs the question: Would these men benefit from hormone replacement?

It's a question KLRI has set out to investigate through its TEAAM (Testosterone's Effects on Atherosclerosis in Aging Men) study.

"We know that testosterone replacement helps build muscle and, at the very least, preserve muscle," explains KLRI Clinical Director Panayiotis D. Tsitouras, MD, who is leading the TEAAM study. "In addition, studies find that it may improve or at least preserve bone density in older men just as estrogen does in older women." Other studies find it may be beneficial in men with chronic heart failure and coronary artery disease. And, of course, there is testosterone's effect on sexuality in older men, with some studies suggesting that age-related declines in testosterone account for about 20 percent of the observed decline in sexuality activity as men age, he says.

So why not just rub some testosterone gel on every man over 60?

"Safety," says Dr. Tsitouras. For, just as estrogen therapy can fuel the growth of very tiny, undiagnosed breast cancers that may never amount to anything, so, too, might long-term testosterone therapy fuel the growth of very tiny, undiagnosed prostate cancers. Some in the field also worry that long-term hormone therapy could stimulate the progression of atherosclerosis through changes in red blood cells. The reality, however, is that no one really knows just *what* the potential risks and benefits of long-term testosterone therapy are in healthy men.

"Testosterone is not the fountain of youth," admits Dr. Harman. "But it may be helpful in aging men. The question is, what is the risk/benefit ratio and how much can you give safely?"

Thus, KLRI, in conjunction with the University of California at Los Angeles, Drew University School of Medicine in Los Angeles, and Boston University are studying the effects of testosterone on 320 healthy men ages 60 to 85 through TEAAM.

Oxidative Stress and Estrogen

What if you could design an estrogen-like molecule with all of the benefits of the real thing—preventing cardiovascular disease, reducing the risk of dementia, maintaining skin tone, urinary and vaginal health—but none of the negatives? One that could be used by men as well as women. Might you have a proverbial anti-aging pill?

Not quite, but it looks like you *could* have a compound that protects against several major age-related diseases, including stroke, Alzheimer's and Parkinson's disease.

To date, KLRI Scientific Advisory Board member James W. Simpkins, PhD, who chairs the department of pharmacology and neuroscience at the University of North Texas Health Science Center in Fort Worth, has tested at least 10 such "non-feminizing" estrogen compounds in animals, finding the molecules act very much like a potent antioxidant in the brain.

Whether the animals received an injection of estrogen-like compounds before or after an induced stroke, the results have been similar: far less oxidative damage than occurred in animals that didn't get the treatment.

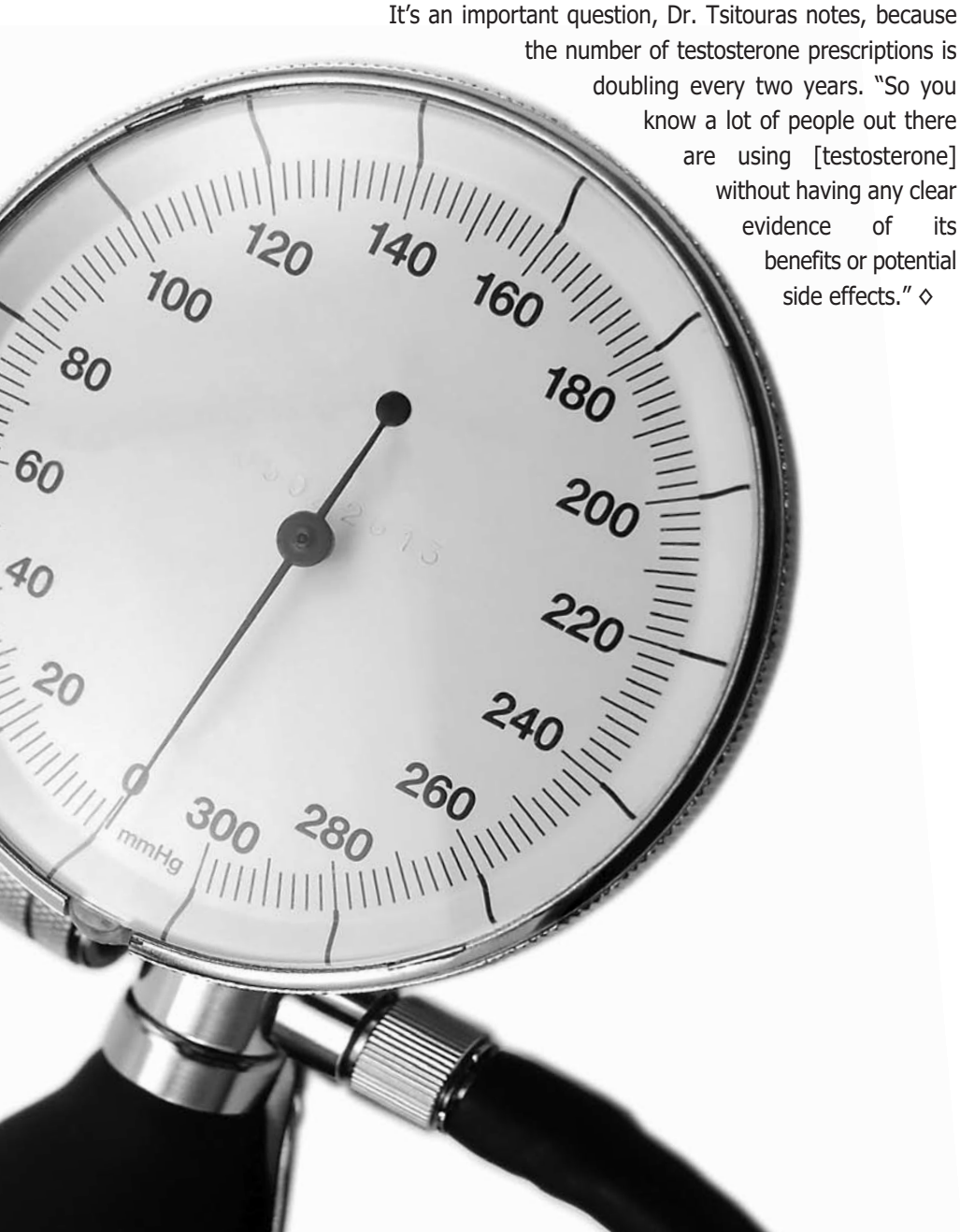
That's important, because much of stroke-related damage occurs after the initial blood clot, when immune-system cells rush in to repair the clot-induced damage. That, in turn, leads to significant inflammation and oxidative stress, further damaging brain neurons.

Testosterone *continued*

Researchers will track the development of atherosclerosis in the men as well as their levels of lean body mass, muscle function, cognitive function, and health-related quality of life to determine testosterone's effects. They will also track levels of prostate-specific antigen (PSA), a marker for prostate cancer.

"And then," says Dr. Tsitouras," we should have a reasonable answer as to the effects of testosterone therapy on heart disease and lipid profiles, as well as body composition, bone density and insulin sensitivity." While the study isn't large enough to answer the question about testosterone therapy and prostate cancer, it should provide enough clues to, hopefully, convince the National Institutes of Health (NIH) to fund a much larger study.

It's an important question, Dr. Tsitouras notes, because the number of testosterone prescriptions is doubling every two years. "So you know a lot of people out there are using [testosterone] without having any clear evidence of its benefits or potential side effects." ◊



Oxidative Stress *continued*

Estrogen likely has numerous effects in the brain beyond its antioxidative effects, says Dr. Simpkins. One appears to be its interaction with mitochondria, the energy generators in cells. "We have experimental data showing that [estrogen-like compounds] enable mitochondria to make usable forms of energy without producing as many radical oxygen species," he says. Radical oxygen species are molecules responsible for oxidative damage.

There is also evidence that estrogen protects against dementia. One major study found that women who have their ovaries removed before menopause—effectively shutting down most of their body's production literally overnight—have a 46 percent increased risk of dementia compared to women who enter menopause naturally (and thus have longer exposure to estrogen).[9] Early surgical menopause also increases the risk of Parkinson's disease.[10] Studies also find lower rates of Alzheimer disease in women who start taking hormone therapy soon after menopause.[11,12] And other research finds that "pulses" of estrogen every few weeks can boost cognitive performance and brain function in areas that control memory in monkeys, and that estrogen can reduce deposits of beta amyloid, plaques commonly found in Alzheimer's patients.[13,14,15]

Hence the importance of estrogen-like compounds. Next steps: taking one or more of these compounds into human trials. ◊

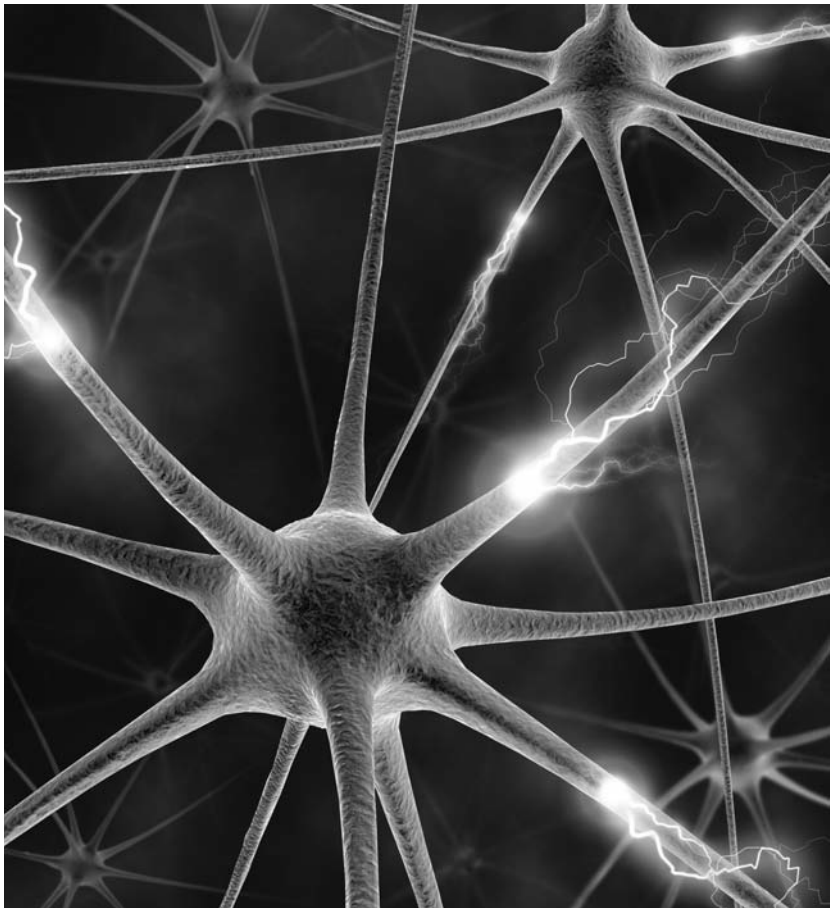
Human Growth Hormone:
The Possibilities

Forget about baseball players and body builders. The real possibilities for human growth hormone (hGH) may lie in improving quality of life and health of people as they age. But it's a big "maybe."

Growth hormone is secreted by the pituitary gland. One of its primary functions is to stimulate growth in children, primarily through the production of another hormone called insulin-like growth factor-I (IGF-I). The two also play a role in bone health, the ability of cells to use glucose for energy, insulin production and overall immune system health.

Like estrogen and testosterone, hGH levels also decline with age, with people in their late 60s and 70s often showing significantly reduced levels.[22] But are those lower levels related to the increased levels of insulin resistance, cardiovascular disease, muscle and bone loss seen in older adults?

The simple answer is, we don't know.



Recombinant Human Growth Hormone:
Not Ready for Prime Time

An estimated 20,000 to 30,000 people used growth hormone as an anti-aging therapy in 2004, a more than 10-fold increase over its use in the mid-1990s. But before you order it online or try and find a doctor who will prescribe what some call the "sweet syringe of youth," you should know that such use can be extremely dangerous, particularly in older people.[24]

Plus, a review of numerous studies of its use in healthy elderly individuals found no evidence of improvement in the ability to exercise, bone mineral density, cholesterol or triglyceride levels, or blood sugar, or insulin levels.[24] In fact, all it likely does is suppress an individual's own secretion of hGH. And recombinant hGH isn't cheap. It can cost $10,000 a year or more.

So if there's no evidence it works, and a high risk of dangerous side effects, why do so many people continue to inject it?

"Myths and legends die very hard," says Laurence S. Jacobs, MD, a member of KLRI's Scientific Advisory Board who has spent decades researching hormone-related topics. "And the absence of controlled trial data to support those myths, or even the presence of good controlled trial data, frequently has very little effect because people are always looking for magic bullets."

What is clear, however, is that when it comes to human growth hormone—at least for now—they need to keep looking. ◊

Human Growth Hormone *continued*

Most studies using supplemental hGH, called recombinant human growth hormone (rhGh), were conducted in people who didn't produce enough of their own hGH because of an underlying pituitary-related disease. The few studies conducted in healthy older individuals, including one by KLRI and the National Center for Complementary and Alternative Medicine at NIH, showed few benefits and significant side effects.

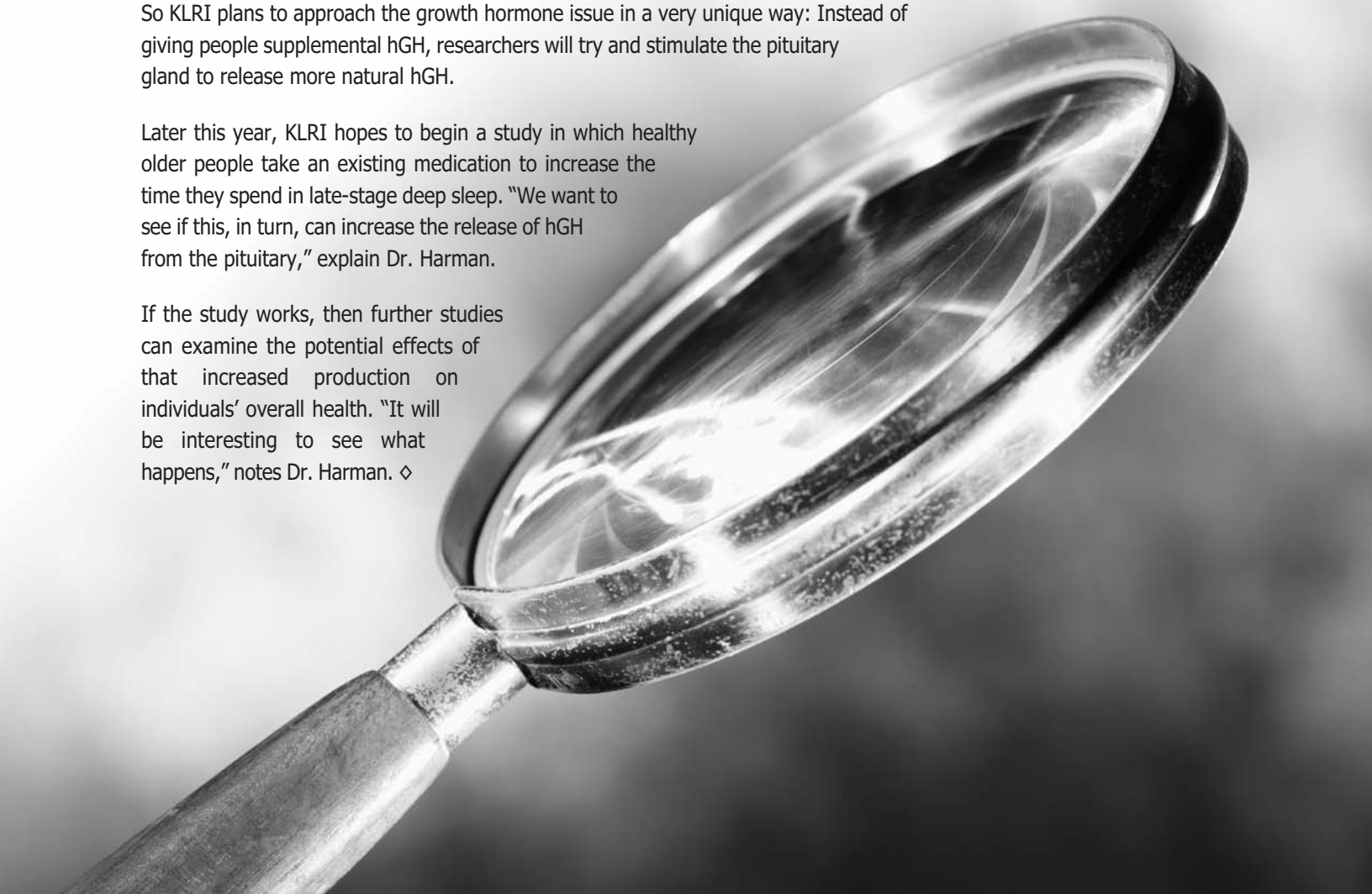
In the NIH study conducted by Dr. Harman, for instance, 131 healthy people over age 65 received rhGh with or without estrogen/progestin (in women) or testosterone (in men).[23] In women, the combination of rhGH alone or with estrogen reduced total body-fat levels, while in men the combination of rhGH and testosterone, as well as testosterone alone, reduced body-fat levels. However, the women had significant swelling and joint pain, while the men experienced symptoms of carpal tunnel and joint pain. Several men also developed diabetes and glucose intolerance. Similar studies found headaches, joint swelling, joint pain and bloating in healthy older adults receiving rhGH.

Another challenge with using hGH in healthy individuals is that, unlike estrogen and testosterone, hGH must be injected daily, something few people want to do if they don't absolutely have to. Even more challenging is the fact that growth hormone is secreted in the body in pulses throughout the day and, especially, at night during the deep sleep stages (one reason levels drop in older people may be because many older people don't reach those deep sleep stages). With an hGH injection, however, you get a large increase that lasts for several hours before gradually dropping. That "unnatural" boost of growth hormone could help explain the side effects and lack of benefit seen when the hGH is given to healthy people, says Dr. Harman.

So KLRI plans to approach the growth hormone issue in a very unique way: Instead of giving people supplemental hGH, researchers will try and stimulate the pituitary gland to release more natural hGH.

Later this year, KLRI hopes to begin a study in which healthy older people take an existing medication to increase the time they spend in late-stage deep sleep. "We want to see if this, in turn, can increase the release of hGH from the pituitary," explain Dr. Harman.

If the study works, then further studies can examine the potential effects of that increased production on individuals' overall health. "It will be interesting to see what happens," notes Dr. Harman. ◊



NUTRITION and Aging

If, indeed, you are what you eat, then what is the potential of nutritional interventions to reduce the risk of disease and frailty as we age? Tremendous, it turns out.

Let's start with insulin resistance. As we age, we are much more likely to develop insulin resistance, regardless of our weight. The condition occurs when cells become resistant to insulin, a hormone required to "unlock" cells to allow glucose to enter. Cells need glucose to create energy. But if cells remain closed to glucose, sugar and insulin build in the blood, triggering inflammatory processes that can damage cells lining those blood vessels, increasing the risk of atherosclerosis and other conditions. Insulin resistance is also a precursor to type 2 diabetes.

Type 2 diabetes is no longer relegated to older adults, or even adults. In the past 10 years, the number of children and adolescents with the disease has skyrocketed, an increase experts like Arline Salbe, PhD, blame on our increasingly unhealthy diets and the concomitant explosion of overweight children. This is particularly troubling to Dr. Salbe, senior clinical research fellow at KLRI, because of the years she spent studying the disease in Pima Indians, a group of Native Americans that has the highest rates of diabetes in the world. "I know that the earlier diabetes begins, the more serious the complications," she says. Complications could include blindness, nerve damage, cardiovascular disease, kidney failure, cognitive impairment and weaker muscles.[25,26] Weak muscles might not sound like a big deal compared to heart disease, but it sets you up for frailty during older age, which would severely limit your independence and reduce your overall quality of life.

So KLRI researchers are beginning to explore nutritional interventions in aging, particularly diabetes. Because diabetes is linked to high rates of inflammation, KLRI wants to examine the potential of dietary manipulation to reduce inflammation.

The first such study focused on omega-3 fatty acids. These "healthy" fats, found primarily in fatty fish like salmon and tuna and in some seeds, reduce the risk of sudden cardiac death, have significant anti-inflammatory activities, and are being used to reduce inflammation in diseases like rheumatoid arthritis and Crohn's disease.[27]

Yet the typical American diet is low in omega-3s and high in other unhealthier fats such as trans-fatty acids and saturated fats. It is also too high in polyunsaturated fats like omega-6 fatty acids, found in vegetable oils. Omega-6, unlike omega-3, can negatively affect immune-system functioning and increase inflammation, blood clotting and plaque development.

Omega-3 and omega-6 fatty acids also contribute to the makeup of cell membranes. As we age, cells gradually lose their ability to receive signaling messages from other cells or from the environment, a factor linked to the development of many age-related diseases. Higher levels of omega-3 fatty acids compared to omega-6 fatty acids help membranes retain their "fluidity," which, in turn increases their ability to receive signals required for proper functioning.

So, KLRI researchers wanted to know, could increasing levels of omega-3 fatty acids in the diet improve cellular signaling? The preliminary answer appears to be. . . maybe.

Researchers had healthy men and women ages 60 to 75 eat a normal diet low in omega-3 fatty acids followed by a diet high in those healthy fats. The participants got their omega-3 fatty acids by eating fatty fish every week and taking fish oil each day.

After the high omega-3 fatty acid diet, participants' red blood cells had twice as much omega-3 in their membranes as omega-6. Plus, participants showed significantly improved insulin sensitivity and reduced markers of inflammation.

Next step: Explore the effects of the same dose of omega-3 fatty acids on people with impaired glucose tolerance or early diabetes. "We expect to see similar or better effects as in the previous study on healthy people," predicts Dr. Tsitouras. ◊

Is There An Anti-Aging Diet?

Scientific Advisory Board member Judith Wood Hallfrisch, PhD, would say yes. Dr. Hallfrisch spent most of her career with the United States Department of Agriculture (USDA) researching the effects of diet on health, particularly the health of older people. Based on her findings, an anti-aging diet would include:

- Plenty of whole grains, particularly barley and oats, to reduce cholesterol and blood pressure levels, and to increase glucose tolerance, reducing the risk of diabetes.[28,29]

- Lots of fruits and vegetables, which, among other things, increase levels of "good" cholesterol and, together with a diet low in saturated fat, can significantly reduce the risk of early death.[30,31]

- A minimum of saturated fats, the type of fat found in animal-based foods like meat and dairy.

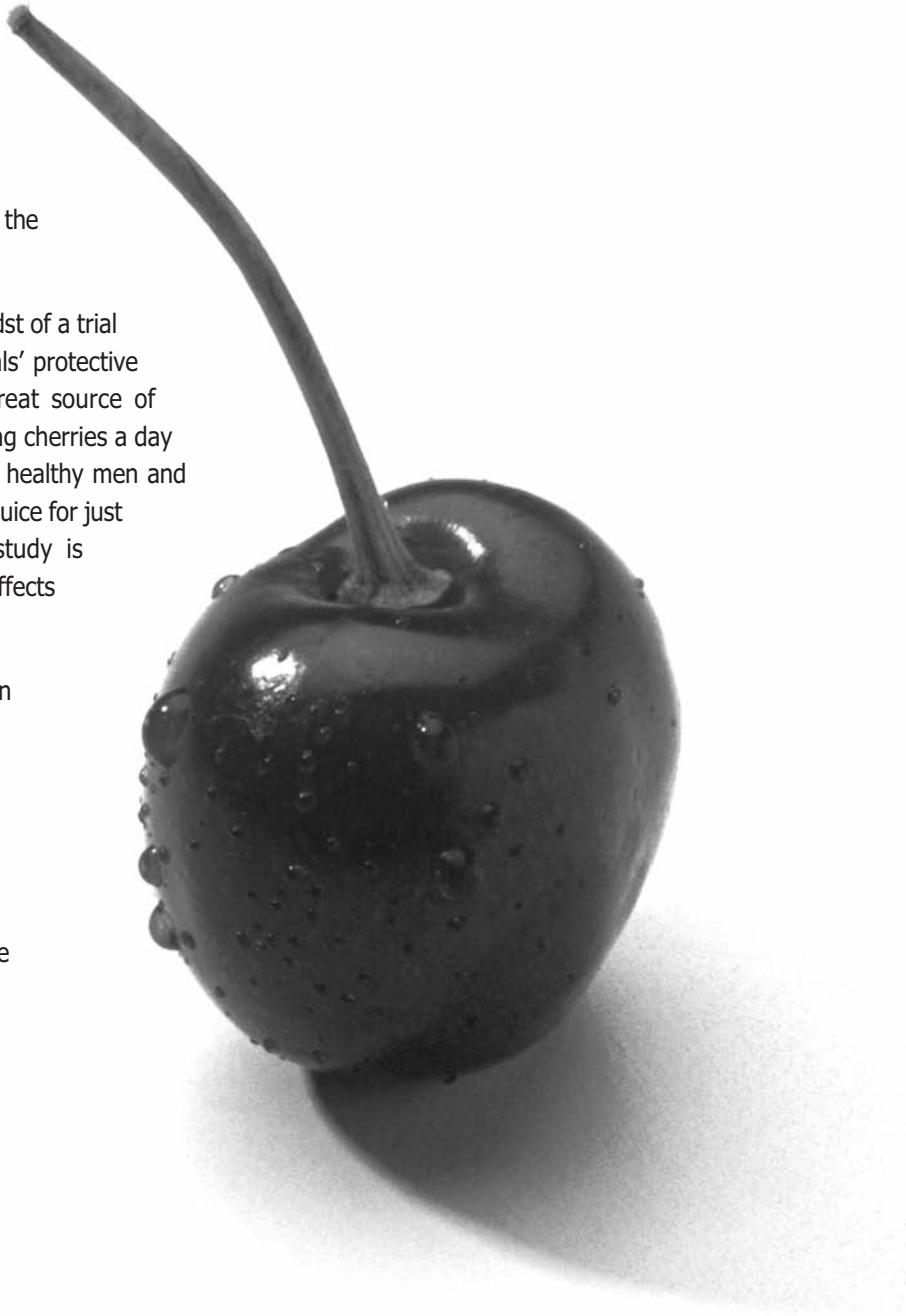
- Little-to-no high-fructose corn syrup, which her work found is more likely to increase triglyceride and cholesterol levels as well as blood pressure, particularly in people who already have problems with blood pressure, cholesterol levels, and blood glucose, or in postmenopausal women.[32] ◊

Using Nature's Antioxidants to Fight Oxidative Stress

As KLRI studies show, and as discussed earlier in this report, our innate ability to protect ourselves against damaging oxidative stress weakens with age. Yet many foods, particularly fruits and vegetables, are high in antioxidants that could, presumably, improve that response. So it makes sense to ask: Would adding foods high in antioxidants improve the antioxidant response in older people?

We should know soon. KLRI investigators are in the midst of a trial evaluating the effects of tart cherry juice on individuals' protective response to high oxidative stress. Cherries are a great source of antioxidants, with studies finding that just 45 sweet Bing cherries a day is enough to reduce levels of inflammatory markers in healthy men and women. A study of young people found drinking cherry juice for just four weeks reduced oxidative DNA damage. This study is designed to see if the same juice might have similar effects in older people.

Researchers are using the perfusion test described on page 8 to induce oxidative stress. They will measure individuals' response to the stress before drinking the cherry juice, then again after drinking either 16 ounces a day of the juice or a placebo juice for 14 days. After participants follow their regular diet (with no juice) for four weeks, the test will be repeated, only this time those who originally got the juice will now get the placebo and vice versa. ◊



LOOKING Forward

Revealing the biological underpinnings of aging is like peeling an onion: as one layer is removed, four more are identified. But through the careful scientific processes used by researchers at KLRI and elsewhere, the core of the question "What is age?" is gradually being revealed.

What this means for the hundreds of millions of Americans who are moving into their later years cannot be underestimated. For the first time in human existence, they—and we—may be facing a life that doesn't end in disability and pain, in dependence and sickness, but that continues full of energy and learning until, finally, we reach a natural, peaceful end to our finite life span. ◊

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Kronos Longevity Research Institute

Who We Are

Kronos Longevity Research Institute (KLRI) is a not-for-profit 501(c)(3) organization that conducts state-of-the-art clinical translational research on the prevention of age-related diseases and the extension of healthier human life. Translational research is the critical link between findings from the basic research laboratory and corresponding improvements in clinical care.

Mission: KLRI is dedicated to understanding the human aging process and preventing age-related disease. KLRI conducts and fosters research that moves basic discoveries into clinical practice and communicates our research results to scientific and healthcare professionals and to the public so that people may enjoy longer and healthier lives.

Research Focus:
KLRI's research team has identified five areas of concentration that promise to yield the greatest progress in helping people live healthier lives in their later years:

- **Damage to cells and tissues by oxidative stress** — the cumulative effect of reactive oxygen on the body's cells is a key mechanism of the aging process and plays an important role in diseases such as hypertension, heart disease (hardening of the arteries), cancer, and perhaps Alzheimer's disease. To date, the ways to measure the damage of oxidative stress on the body have not been established.

- **Cardiovascular health and hormonal balance** — to help physicians and their patients understand the benefits and risks of hormone therapy (particularly estrogen and testosterone).

- **Nutritional studies** — which will replace anecdotal, hit-or-miss evidence on nutrition and the benefits of dietary supplements for mid-life and aging patients with a set of specific and scientifically documented recommendations.

- **Age-related changes in body composition** — that lead to muscle loss (sarcopenia) and lower functioning of the body's organs, which may be postponed, ameliorated or prevented through sound translational research.

- **Changes to the body's immune system** — which cause it to attack the body's own cells and tissues or to lose its ability to ward off infections. Rejuvenation of the aging immune system may prevent, cure or arrest such diseases as type1 diabetes, rheumatoid arthritis, autoimmune thyroid failure, Parkinson's and some cancers.

Funding: KLRI depends on funding from private foundations, government funding and individual donors.

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