

KLRI

Gray
is the new
Gold

STATE OF THE SCIENCE
TWO THOUSAND NINE

OPTIMISM
IN AGING RESEARCH

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EXECUTIVE SUMMARY

This State of the Science Report was produced just after the new President took office, ushering in an era of optimism and hope within the scientific community. As you read through the report and recognize the potential of the research being conducted within the longevity field, we hope you, too, will feel the same.

Among the research described in this year's State of the Science Report:

The Longevity Dividend. The Longevity Dividend is based on the theory that if we can intervene scientifically to slow the aging process and delay the onset of age-related diseases, trillions of dollars now spent on health care could be redirected to schools, energy, jobs, infrastructure—the “dividend.” A group of leading scientists hopes to convince the federal government to change medical research funding from its focus on individual diseases to a focus that recognizes the importance of research into the underlying biology of aging. Only then, they contend, can the Longevity Dividend become a reality.

Oxidation, inflammation and insulin resistance. These are the “three horseman of aging,” believed to underlie nearly all age-related diseases and processes. Current work at KLRI includes a study to see if insulin sensitizers can reduce inflammation and oxidative stress. Elsewhere in the country, researchers are investigating the role of nutrition in stemming oxidation, inflammation and insulin resistance, finding that powerful plant-based antioxidants called polyphenols can prevent and reverse the effects of aging on memory brain cells and function.

Telomeres and insulin resistance. Telomeres are caps on the end of a cell's chromosomes that help keep chromosomes stable, just as the cap on a pen prevents ink from leaking. With time, however, the telomere shrinks. The shorter the telomere, the worse the cell functions and the closer it is to death. New research suggest that in addition to age, being overweight or obese can wreak havoc on telomere length even in your twenties, thanks to insulin resistance.

Physical fitness and exercise training. To learn more about the benefits of exercise in preventing age-related declines, KLRI researchers have begun a study to measure the response of fit and unfit older men and women to two acute stressors: a blood pressure test, which increases oxidative stress, and a psychological test, which increases neuroendocrine stress, releasing inflammatory chemicals. Researchers will also look for any link between oxidative stress and neuroendocrine responses.

Calorie restriction. Numerous studies have found that restricting an animal's calories by 25 to 30 percent can extend their lifespan. A five-year trial called CALERIE (Comprehensive Assessment of Long-term Effects of Restricted Intake of Energy), which involves 250 healthy volunteers ages 25 to 45 assigned to either restrict their calories by 25 percent or be part of a control group, has already produced some interesting data. For instance, calorie restriction reduces insulin levels, core body temperature, energy expenditure and DNA damage. It can also increase cellular resistance to stress proteins.

Hormones and aging. While the Kronos Early Estrogen Prevention Study (KEEPS), designed to evaluate the effect of estrogen on heart disease in younger, postmenopausal women, continues, ancillary studies underway could provide interesting data on other topics. These include menopause and age-related skin changes and the effects of estrogen on blood cell function and the formation of blood clots. Meanwhile, KLRI's TEAAM (Testosterone Effects on Atherosclerosis in Aging Men) completed recruitment and is engaged in the research necessary to track the effect of supplemental testosterone on a variety of age-related markers.

While both trials will examine the role of hormones in cognitive function, research published this year from other studies found no effects from either a low dose of estrogen or supplemental testosterone on cognition.

Vitamin D. Vitamin D is turning out to be a critically important vitamin for all aspects of health, particularly those related to aging. Low levels have been linked to urinary incontinence, problems swallowing (dysphagia), breathing ability (increasing the risk of pneumonia), age-related macular degeneration, dementia, influenza and several cancers, including colon, breast and prostate.ⁱ Yet 40 to 100 percent of elderly men and women living in the community, and more than half of postmenopausal women taking osteoporosis medication, have clinically low levels of vitamin D.

KLRI research.

- The results of a KLRI study published in the *Journal of the American Aging Association* this summer showed that statin use in older adults does not negatively affect aerobic exercise or high-intensity weight training.
- A study published in the journal *Hormonal and Metabolic Research* in March 2008 showed that after 10 weeks on a diet high in omega-3 fatty acids, participants demonstrated significantly greater insulin sensitivity and lower levels of some circulating inflammatory markers. They also released fewer fat molecules that contribute to inflammation and oxidation.ⁱⁱ
- A small pilot study will investigate the effects of vinegar on hunger, fullness and glucose absorption over a three-hour period.

All in all, 2008 was a busy and productive year for biogerontologists everywhere and for KLRI scientists. We look forward to further developments and to keeping you up to date on the most exciting findings in age-related science.

INTRODUCTION

As we look back at 2008 and forward to 2009, we at the Kronos Longevity Research Institute (KLRI) feel optimistic, despite the gloomy economy and global uncertainty. We are optimistic because of the new administration's commitment to science and scientific research, and because of the breadth and quality of scientific research into longevity and aging issues throughout the country.

In this State of the Science Report, we introduce the concept of the Longevity Dividend, which could prove invaluable as this country begins work on the healthcare system of the future. We also update you on efforts to unravel the complex interactions between the "vicious triad of aging"—oxidative stress, inflammation and insulin resistance—and age-related disease; and, of course, fill you in on our progress here at KLRI, where we're proud to announce the receipt of a \$100,000 National Institute on Aging (NIA) grant to explore interactions between fitness and aging on stress resilience. ◇

THE LONGEVITY Dividend

Ever heard of the Longevity Dividend?

It's a theory that says we hope to intervene scientifically to slow the aging process, which will also delay the onset of age-related diseases. Delaying aging just seven years would slash rates of conditions like cancer, diabetes, Alzheimer's disease and heart disease in half. That's the longevity part.

The dividend comes from the social, economic, and health bonuses that would then be available to spend on schools, energy, jobs, infrastructure—trillions of dollars that today we spend on healthcare services. In fact, at the rate we're going, by the year 2020 one out of every \$5 spent in this country will be spent on healthcare. Obviously, something has to change.

Enter the Longevity Dividend. The Longevity Dividend doesn't suggest that we live longer; instead, it calls for living *better*. The idea is that if we use science to increase healthspan, not lifespan. In other words, tomorrow's 50-year-old would have the health profile of a 43-year-old.

It might sound like science fiction, but, in fact, it's quite possible. We're already doing it in some animal models using genetic and dietary interventions, techniques related to what scientists call "the biology of aging."

Getting there in humans, however, means embracing an entirely new approach to our thinking about disease and aging, and how we conduct scientific research into the two. ◇

GETTING SCIENTISTS' Attention

A group of eminent researchers first proposed the Longevity Dividend in a 2006 article published in *The Scientist*. The authors, S. Jay Olshansky, PhD, professor of epidemiology and biostatistics at the University of Illinois in Chicago, Daniel P. Perry, executive director of the Alliance for Aging Research in Washington, DC, Richard A. Miller, MD, PhD, professor of pathology at the University of Michigan in Ann Arbor, and Robert N. Butler, MD, president and CEO of the International Longevity Center in New York, intended their essay to be a "general statement to scientists" about the need for a paradigm shift in the way we think about aging and disease.¹

The researchers also met with U.S. senators who served on the Senate committee that oversaw the budget for the National Institutes of Health (NIH). "We told them we believed that a new way was available to us to improve health in this century, but it was an approach that was fundamentally different from the approach we had been taking," recalls Dr. Olshansky. Instead of focusing on individual diseases, the researchers said, significantly more funds should be shifted to research on the biology of aging so we could unravel the underlying pathophysiological processes that eventually result in cellular damage and lead to age-related diseases.

The scientists were successful—to a point. The fiscal year 2008 Labor/Health and Human Services Appropriations bill *did* include language acknowledging the importance of holistic research into the underpinnings of aging itself:

"The Committee commends the (National Institute on Aging [NIA]) for work it has done to improve understanding of the biological factors that regulate the processes of aging. These new discoveries have led many scientists to believe that it may become possible to postpone the onset of a wide range of fatal and disabling diseases, in a coordinated fashion, by retardation of the aging process. It is widely understood that

chronic illness is a powerful driver of medical costs, which in the United States are expected to reach \$16 billion annually by 2030. To alleviate this financial burden and to develop interventions that can extend health and longevity, the Committee urges the NIH to increase dramatically its annual investment in the biological basis of aging."

Unfortunately, the NIA still received just 3.5 percent of the nearly \$30 billion NIH budget in fiscal year 2008 (see table on page 6). Compare that to the National Cancer Institute, which received 16.1 percent of the funding, the largest slice of the pie. Yet if we spent more to unravel the cellular secrets of aging, contended Dr. Olshansky and his colleagues, we wouldn't need to spend so much on cancer and other diseases of aging because fewer people would develop them.

To get this message across to clinicians as well as scientists, in July 2008 the researchers published another essay, this time in *The British Medical Journal*. "A New Model of Health Promotion and Disease Prevention for the 21st Century" contended that the effectiveness of medical research worldwide "will become limited unless there is an increased shift to understanding how aging affects health and vitality."²

For instance, the report noted, since most people have more than one chronic disease in the final third of their lives, curing any of the major fatal diseases would "have only a marginal effect on life expectancy and the overall length of healthy life."

"We are ultimately talking about the best form of prevention you can have," said Dr. Olshansky of work to understand the biology of aging. "And this ultimate method of prevention will carry with it significant bonuses or dividends. People will be healthier longer so there will be many opportunities to spend money on things *other* than healthcare." ◇

National Institutes of Health Appropriations: Fiscal Year 2008³

Total: \$29.46 billion

National Cancer Institute	\$4.81 billion
National Institute of Allergy and Infectious Diseases	\$4.56 billion
National Heart, Lung and Blood Institute	\$2.92 billion
National Institute of General Medical Sciences	\$1.94 billion
National Institute of Diabetes and Digestive and Kidney Diseases	\$1.86 billion
National Institute of Neurological Disorders and Stroke	\$1.54 billion
National Institute of Mental Health	\$1.40 billion
National Institute of Child Health and Human Development	\$1.25 billion
National Center for Research Resources	\$1.15 billion
Office of the Director	\$1.11 billion
National Institute on Aging	\$1.05 billion

OXIDATION, INFLAMMATION AND INSULIN Resistance

Oxidation, inflammation and insulin resistance. Think of them as the Three Horsemen of aging. Inextricably linked, each causing the other, all related to age-related disease and morbidity.

First, a brief review. Oxidation, or oxidative stress, results from the activities of molecules called free radicals. These are formed as byproducts as cells create energy and, to a lesser extent, also come from exposure to environmental factors such as cigarette smoke and radiation. They're kind of like the smoke and ash that come out of power plants turning coal into energy. Just as all that pollution damages the environment, so, too, can free radicals damage nearby cells. Enough damage, and the cell gets sick, doesn't work properly and the next thing you know *you* get sick.

Luckily, power plants have scrubbers and your body has antioxidants. These molecules live for the moment when they can scoop up free radicals and render them harmless. Antioxidants include nutrients like vitamins A, C and E; minerals like selenium; and amino acids like glutathione. Where do these come from? Mainly from our diet, of course. Remember this; we'll get back to the whole diet thing shortly.

Now we come to inflammation. You see inflammation in action every time you cut your hand or get a fever. When your body senses any kind of threat, whether from a virus, a bacteria or a slipped bagel knife, immune system cells rush in to repair the damage. Unfortunately, some of these cells bring more of a slash-and-burn approach than a targeted approach, leaving collateral damage in their wake.

That's because certain immune system cells release inflammatory chemicals such as cytokines and leukotrienes. These chemicals are good—in the short term. They signal more targeted immune cells to join the battle, directly neutralize certain pathogens, make blood vessels permeable so other immune system compounds can quickly reach trouble spots, and increase blood flow to the injured area, bringing extra oxygen and nutrients.

In the process, however, levels of cholesterol and other blood lipids rise, glucose production increases, stress hormones like adrenaline and cortisol are released and insulin sensitivity declines. Not only that, but all this activity calls for extra cellular energy, which means increased oxidative stress.

Now, none of this is a problem if you're dealing with an acute injury or infection that is quickly vanquished. But if you're overweight or obese, have a chronic infection (even one with no overt symptoms) or autoimmune disease, are exposed to toxins (think smoking) or are under chronic stress, you set up a continual loop of inflammation and oxidative stress that keeps your body on constant high alert, triggering, among other things, chronic insulin resistance.

Insulin resistance occurs when cells are no longer fully receptive to insulin, the hormone that "unlocks" a cell so glucose can get in and provide the fuel for energy. If the cell won't open to glucose, it builds up in your blood. Your pancreas thinks there isn't enough insulin around to get the glucose into the cells, so it churns out more. Now you have

high levels of both insulin *and* glucose, which, like a mob waiting to get into a department store the day after Thanksgiving, means trouble. Only in this case it's not the threat of violence, but the threat of inflammation and oxidation that is the worry.

The more we learn about insulin resistance, the more convinced we are that it may well be one of the most important biomarkers of aging, said S. Mitchell Harman, MD, PhD, KLRI director and president. "That's why we're so interested in learning what can be done to ameliorate the triad of oxidative stress, inflammation and insulin resistance." Thus, KLRI is instituting several new studies around this conundrum. One, for instance, will look at how a drug used to improve insulin resistance in people with diabetes also affects inflammation and oxidative stress. It may be that insulin sensitizers will turn out to be the first true "anti-aging" drugs.

Other researchers are investigating the role of nutrition in stemming oxidation, inflammation and insulin resistance. James Joseph, PhD, chief of the Neuroscience Laboratory at the Human Nutrition Research Center on Aging at Tufts University in Boston, focuses on nutritional interventions to slow or prevent brain aging and cognitive decline.⁴

"As we get older, we are less able to deal with oxidative and inflammatory stressors," he explains. The impact of oxidation and inflammation is particularly damaging in the brain, leading to age-related cognitive decline, dementia, and Alzheimer's and Parkinson's diseases.

Dr. Joseph's team has shown that not only can powerful plant-based antioxidants called polyphenols prevent and reverse the effects of aging on memory brain cells and function, but they may also *improve* signaling between brain neurons and enable brain cells to regenerate themselves, a process called *neurogenesis*. This may be particularly important when it comes to preventing or reversing brain-related conditions like Alzheimer's and Parkinson's. You've probably heard of at least one polyphenol: resveratrol, a compound found in red wine and grapes (more on resveratrol to the right). It belongs to the stilbene family of polyphenols.

In 2008, Dr. Joseph and his team published data showing that one way in which polyphenols protect brain cells from oxidative and inflammatory stress is by restoring full function to the cellular membrane, the thin outer layer of the cell that controls exchange of water, nutrients, ions, and signals between the cells internal machinery and the environment outside the cell. That's critical, since any type of damage to the membrane affects a cell's overall function.⁵

Their research showed that blueberry extract and walnut-based polyunsaturated fatty acids restored the ability of brain cells to shut down oxidative stress signals by preventing the stressor from pulling calcium out of the cell. They also showed that these nutrients appeared to improve communication between brain cells and increased the ability of brain cells to form new connections (plasticity).

The Resveratrol Story

The only way you could have missed the news about resveratrol in the past couple of years was if you had sworn off all newspapers, magazines, and television news shows—the media blitz has been that intense. The news? That resveratrol can increase longevity. Before you could say "Cabernet Sauvignon," the press was speculating about "longevity in a pill" and drugstore shelves were spouting resveratrol supplements.

Ready for a dose of reality?

Resveratrol is a powerful antioxidant. It appears to work much like caloric restriction by activating a protein called SIRT 1 that regulate several processes related to aging, including glucose and insulin production, cancer development and the stability of the genome, or DNA, when cells divide.

Numerous studies find that resveratrol extends lifespan in worms, fruit flies and other invertebrates^{7,8,9,10} and can slow age-related changes in mice.^{11,12} While earlier studies suggested that resveratrol might, in fact, also increase lifespan in mice, this year two published studies showed that mice fed resveratrol supplements lived no longer than mice who didn't get the supplements.^{13,14}

The studies did, however, find that the cells of resveratrol-fed mice were less likely to show DNA mutations. These mice also did not show genetic changes associated with cardiovascular and muscle aging, or any age-related cardiovascular problems.

Resveratrol *continued*

However, unlike caloric restriction resveratrol did not prevent cancer or reduce levels of insulin-growth-factor I, low levels of which are linked to longevity.¹⁵

Nonetheless, the studies were still good news. After all, as we noted in the article on the Longevity Dividend on page 4, healthspan may, in many cases, be more relevant than lifespan.

These studies also help confirm what we've suspected for a while: that resveratrol may not be powerful enough to exert the same effects in mammals as it does in invertebrates like worms and flies. That's why researchers are searching for similar, more powerful compounds.

You can see this basic science translated into action in old mice. When Dr. Joseph's team supplemented the diets of these mice with walnut oil, their spatial learning ability and memory significantly improved.⁶

Basically, says Dr. Joseph, his studies show that these powerful plant chemicals "can get the machinery" working again.

This does *not* mean, however, that you should start popping antioxidant supplements like resveratrol as if they were M&Ms. "Forget the pills," said Dr. Joseph. "There is no magic bullet here." Although he does take supplements, he says the most important component when it comes to protecting against oxidation and inflammation is a healthy diet packed with fruits, nuts, and vegetables representing every color of the rainbow.

"To eat a bad diet and take supplements to make up for it is like putting lipstick on a pig," he said. ◇



Telomeres and Insulin Resistance

Telomeres are caps on the end of a cell's chromosomes that help keep chromosomes stable, just as the cap on a pen prevents ink from leaking. Every time a chromosome "unzips" to copy its genetic material for a new cell, the telomere gets a tiny bit shorter. The shorter the telomere, the worse the cell functions and the closer it is to death. Studies link shrinking telomeres to age-related conditions such as high blood pressure and high cholesterol, insulin resistance and early death, primarily from infection and cardiovascular disease.¹⁷ But it isn't simply time that shrinks telomeres.

It turns out that being overweight or obese can wreak havoc on telomere length even in your twenties. That discovery comes from the Bogalusa Heart Study, which found that the greater a person's body mass index (a measure of weight in relation to height) the shorter their telomeres.¹⁸ The primary reason for the link between weight and telomere length? Insulin resistance.

Extra fat not only increases oxidative stress but central body fat—the kind that collects around your middle—releases inflammatory factors – chemicals that cause inflammation. The inflammation and oxidation contribute to insulin resistance. In addition, higher inflammatory factor levels coupled with the increased blood supply needed to support all that extra fat means white blood cells turnover more often. And since telomeres shrink every time a cell divides, you can see why overweight people have shorter telomeres, regardless of their age.

Although we don't know the precise mechanism underlying the shortened telomere in overweight/obese young people, it's pretty clear that it is somehow related to excess weight—another reason to push back from the dinner table and skip the seconds. ◇

Resveratrol *continued*

Dr. Joseph's lab, for instance, has found that pterostilbene, a cousin of resveratrol also found in berries, grapes and red wine, might be just as, if not more, effective. When researchers tested seven "stilbene" compounds in cell cultures, including resveratrol and pterostilbene, they found pterostilbene was most effective at preventing oxidative stress. Researchers then fed old mice pterostilbene supplements and found the higher the amount of pterostilbene, the more the mice's working memory improved (as measured by their performance in the classic mouse maze).¹⁶

While these compounds may one day be developed into medications to address age-related conditions like diabetes, or even to affect age-related cellular changes, it's too soon to begin popping resveratrol or similar supplements in the hopes of improving your health. But a cup or two of berries each day? Now *that's* a great idea! ◇



PHYSICAL FITNESS and Exercise Training

Been for a run lately? You might try it. There's compelling evidence that people who exercise regularly age better than those who don't. For instance, one study in which nearly 1,500 people were followed for an average of 21 years found exercising just twice a week slashed the risk of Alzheimer's disease in half.¹⁹ Even three bouts of exercise a week over six years can reduce your risk of dementia by a third compared to those who exercise less.²⁰

Other studies find regular exercise can reduce the risk of disability in old age,^{21,22} prevent depression,²³ and boost immunity.²⁴ But perhaps the most important role of exercise is its ability to protect against inflammation, oxidative stress,^{25,26} and psychological stress, all of which are implicated in conditions ranging from abdominal obesity, high blood pressure and cholesterol, insulin resistance^{27,28} and an increased risk for cardiovascular disease, diabetes, atherosclerosis, Alzheimer's disease and depression.^{29,30}

The question is *how* exercise does all of this. To help find out, KLRI researchers have received a grant from the NIA to measure the response of fit and unfit older men and women to two acute stressors: a blood pressure test, which increases oxidative stress, and a psychological test, which increases neuroendocrine stress, releasing inflammatory chemicals. Researchers will also look for any link between oxidative stress and neuroendocrine responses. For instance, people who exhibit the greatest release of stress hormones in reaction to the psychological stressor may also exhibit the greatest cellular damage in response to oxidative stress.

"The results from this study can increase our understanding of *how* regular exercise confers its benefits on the aging process," said Tinna Traustadóttir, PhD, lead researcher on the study.

It will also provide preliminary data for later studies to test the effects of increased physical activity in older people, and evaluate dietary and other lifestyle changes on stress resistance capacity in people who already have a high risk of developing age-related diseases. ◇



CALORIC RESTRICTION in 2008

One of the most active areas of research into longevity focuses on calorie restriction. Numerous studies have found that restricting an animal's calories by 25 to 30 percent can extend their lifespan. Although we don't have any real data in humans yet, there is a five-year trial now in progress called, appropriately enough, CALERIE (Comprehensive Assessment of Long-term Effects of Restricted Intake of Energy). The study, launched in early 2007, involves 250 healthy volunteers ages 25 to 45, who are assigned to either restrict their calories by 25 percent or to be part of a control group. Their experience is expected to provide a plethora of data to help scientists better understand the biologic effects of a reduced-calorie diet in humans.

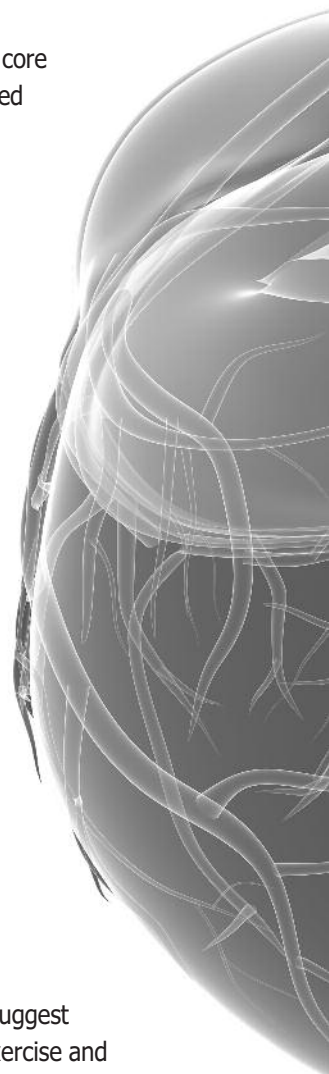
Some early findings have begun to appear in the scientific literature:

- Six months of calorie restriction in slightly overweight participants resulted in lower insulin levels and core body temperature, lower energy expenditure and less DNA damage. Participants also showed improved levels of triglycerides and liver-function markers.^{31,32}
- A group of sedentary men and women ages 50 to 60 who were either normal weight or overweight were randomly assigned to either a calorie-restricted diet or increased exercise. After six months, there was little difference between participants in terms of weight loss, insulin sensitivity and blood glucose levels, nor was there any difference in cardiovascular risk factors. However, the exercisers had increased lung capacity and muscle strength compared with the calorie-restricted group.^{33,34,35}
- Researchers examined cells from CALERIE participants and volunteers in another study called FEAST (in which participants fast every other day for 21 days), subjecting the cells to various stressors and analyzing their genome. They found the cells showed significant declines in their tendency to divide, or proliferate, were more resistant to stress proteins; and showed increased SIRT 1 levels compared to cells collected before any dietary changes. These findings are all relevant to increased health and longevity.³⁶

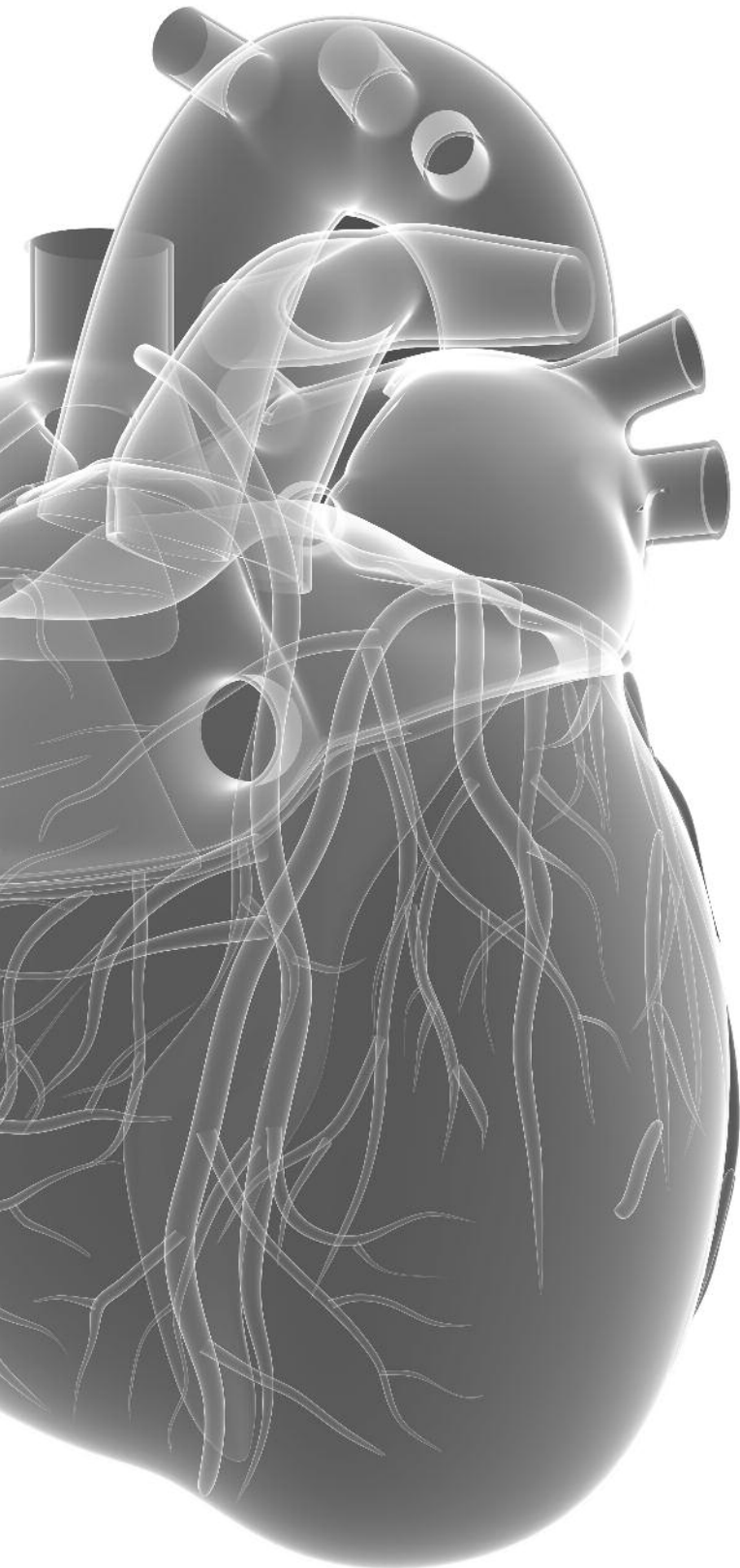
Another study based on white blood cells of 18 participants aged 50 to 60 who either reduced their calories by 20 percent or increased their exercise by 20 percent found both interventions led to substantial yet similar improvements in markers of RNA and DNA damage, likely by reducing oxidative stress.³⁷

So just what does all this mean?

It's still too early to tell, said Dr. Harman. However, the similar effects seen with caloric restriction and exercise suggest that both interventions may be acting through similar pathways. An important question is whether effects of exercise and calorie restriction might be additive or synergistic. ◇



HORMONES and Aging



KLRI investigators continue to make progress on two major studies exploring the effects of estrogen and testosterone on age-related changes in men and women.

Kronos Early Estrogen Prevention Study (KEEPS)

The Kronos Early Estrogen Prevention Study (KEEPS) 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, the arterial disease that causes heart attacks and most strokes. The study is critically needed. In the summer of 2002, results from the Women's Health Initiative (WHI) described a 24 percent increased relative risk of cardiovascular disease in women taking the hormone therapy Prempro, a combination of conjugated equine estrogen and a progesterone called medroxyprogesterone acetate (MPA).³⁸

Because women in the WHI began taking estrogen at an average age of 63—about 12 years later than most women start taking supplemental estrogen — KLRI researchers and others questioned whether the results would be valid in a real-life environment. Thus, KEEPS was born.

Although we don't expect any final results from KEEPS until 2011 or later, a follow-up analysis of the WHI data suggest that the theory behind KEEPS is correct: Starting estrogen therapy earlier in postmenopausal women could provide significant protection against heart disease.³⁹

These analyses found that the timing and duration of hormone therapy, as well as the type (estrogen-only or estrogen + progestin) affected risk, specifically, women who started on estrogen alone between 50 and 59 years of age had a small decrease in risk as did women taking the estrogen/progestin combination if it had been less than 10 years since they reached menopause. In addition, their overall risk of death was somewhat lower. Women taking estrogen-only hormone therapy had a slightly lower risk of heart disease than women taking estrogen/progestin

therapy. After age 59, however, the risk of heart disease increased with age with either treatment. Overall, the risk of heart disease was highest in women starting hormone therapy 20 or more years after menopause and/or who were 70 or older.

"The evidence continues to mount that a woman's age and time since the onset of menopause influences her health outcomes on hormone therapy," said JoAnn Manson, MD, DrPH, a principal investigator with both KEEPS and the WHI. "These findings make KEEPS even more important," she said.

Another advantage of KEEPS is that it provides the first head-to-head comparison of an estrogen patch (transdermal estrogen) with the oral estrogen used in the WHI study (although KEEPS uses lower doses of both oral estrogen formulations than did WHI). KEEPS is also using a natural, or micronized, progesterone formulation rather than the synthetic progestogen used in the WHI.

"There is increasing evidence that the type of progestin used makes a difference (in outcomes)," said Dr. Manson. "Micronized natural progesterone may have advantages over synthetic progesterone, particularly the MPA used in the WHI." For instance, MPA is likely related to the increased rate of breast cancer seen in the estrogen/progestin WHI group. A parallel group of women who supplemented with estrogen alone experienced no increase in breast cancer risk.

Throughout KEEPS, researchers will track the progression of the thickness of the wall of the carotid artery using ultrasound (carotid intima-media thickness) and the build-up of calcium in the coronary arteries of the heart, both markers of atherosclerosis. The data will also be analyzed for any effects of estrogen on women's cognition, including memory and learning ability. ◇

Ancillary Studies Related to KEEPS

The opportunity to collect and examine data from a group of healthy postmenopausal women like those participating in KEEPS has opened up opportunities for studies beyond the initial focus. For instance, a researcher from Yale Medical School is using KEEPS to study the impact of menopause and aging on skin changes in women in early menopause.⁴⁰

Other researchers at the Mayo Clinic in Rochester, MN are examining how estrogen affects blood cell function and the formation of blood clots, or thrombosis, as well as blood vessel response to injury such as an atherosclerotic abscess. Their interest stems from the fact that although heart disease risk increases significantly after menopause, the traditional risk-assessment measure of blood pressure, blood cholesterol levels, age, gender and history does not accurately predict risk in many women, suggesting something else is going on. Plus, they know that estrogen and other hormones affect blood vessel walls and blood cells such as platelets, responsible for clotting.

In one study published in July 2008, the researchers used blood samples from 33 KEEPS participants to search for "microparticles," tiny snippets shed from blood cells and from the cells that line blood vessels (vascular endothelial cells).⁴¹ The researchers then compared the number of microparticles found against the level of coronary artery calcium (CAC) buildup, a marker of heart disease risk. Women with the highest CAC scores also had the highest total number of microparticles, as well as the highest number of particles most likely to clot, or clump.

The researchers theorized that the particles were likely released as a result of injury to blood vessel walls during an inflammatory process. They could indicate a sign of early blood vessel disease or dysfunction, identifying women at highest risk of heart attack, stroke, and other disorders involving thrombosis (blood clotting). Mayo researchers also plan to examine the effects of hormone treatment on circulating microparticles.

Researchers will also study the make-up of the microparticles as part of their efforts to better understand why supplemental estrogen, whether for menopause or birth control, increases the risk of stroke and other clotting disorders (thrombosis). At the same time, they will assess blood platelets, which help blood clot, to better understand their functioning and relation to heart disease and the higher risk of thrombotic events in women taking supplemental estrogen.⁴² ◇

Testosterone Effects on Atherosclerosis in Aging Men (TEAAM)

Testosterone is to men what estrogen is to women—the hormone that defines their gender. As with estrogen, declining testosterone levels in aging men appear related to several age-related changes, including declines in bone mass, muscle mass/strength and physical and sexual function. Low testosterone levels may also play a role in increased weight and abdominal obesity, blood pressure, glucose and insulin levels, and levels of inflammatory markers that suggest an increased risk of heart disease.⁴³

In recent years, studies have found strong links between low testosterone levels and type 2 diabetes, the metabolic syndrome (high blood pressure, insulin resistance, and high risk cholesterol and triglyceride pattern), and heart disease,^{44,45} with the presence of diabetes and coronary artery disease often predicting low testosterone levels.^{46,47} And, as with estrogen, declines in testosterone appear related to cognitive changes and may even be an early sign of Alzheimer's disease risk.^{48,49}

All of which raises the question: Could giving older men supplemental testosterone stem or even reverse some of these age-related conditions? We don't know. But by the time the KLRI's TEAAM study finishes in 2011, we will certainly have some answers.

The TEAAM study, which has already finished recruiting 320 healthy men between the ages of 60 and 85, is designed to track the development of atherosclerosis in men taking supplemental testosterone as well as lean body and fat mass, muscle function, cognitive function and health-related quality of life. Researchers will also track levels of prostate-specific antigen (PSA), a marker for prostate cancer, because of concern that supplementing with testosterone could increase the risk of the cancer. ◇

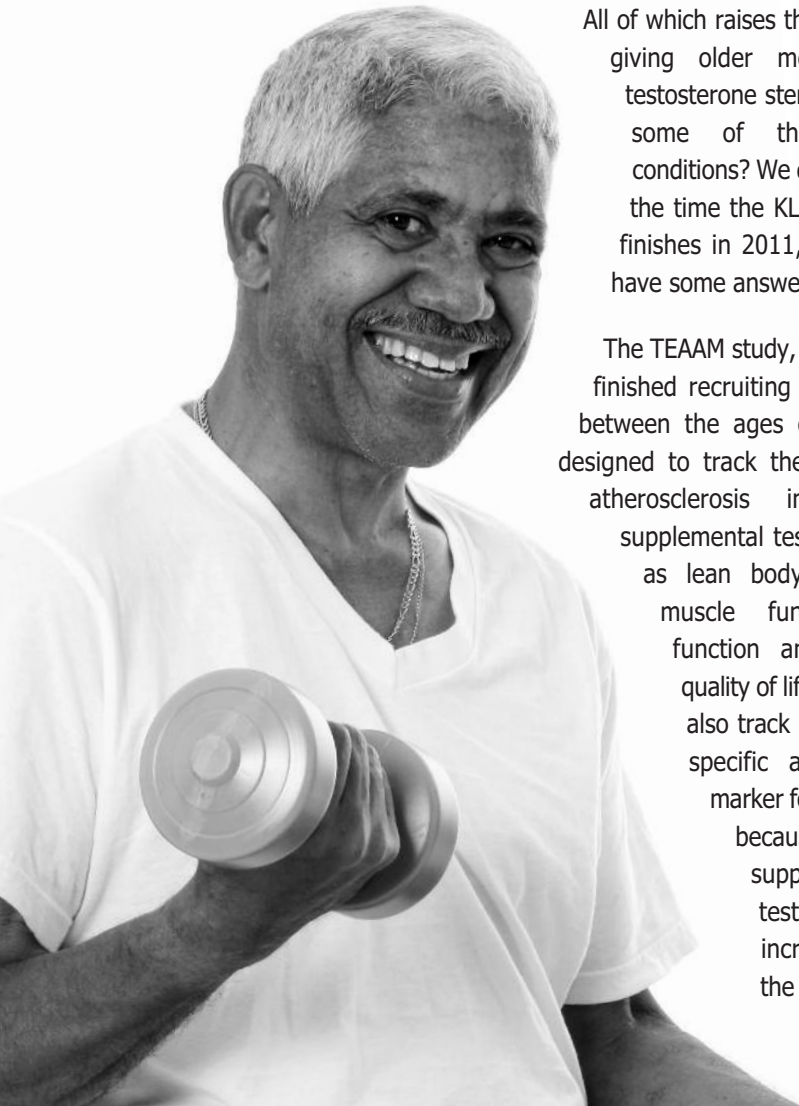
How Old Do You Feel?

Turns out you really *are* only as old as you think! A paper from the long-running Berlin Aging Study published in late 2008 found that people generally feel 13 years younger than their chronological age.⁶⁴

The study is a multidisciplinary investigation of people aged 70 to over 100 who live in former West Berlin. Researchers have followed a core sample of 516 individuals since 1990, examining numerous aspects of their physical and psychological health, social and economic status. In this study, researchers tracked individuals' perceptions of their own age over six years, expecting that as time went on people's "felt" age would more closely match their chronological age.

That only happened, however, if participants had several illnesses and reported less social interaction. In other words, if you were sick and lonely you were more likely to feel your age than if you were healthy and maintained strong social connections. Nonetheless, most were relatively satisfied with their own aging throughout the six-year period.

That's great, because other work shows that the younger you feel and the more satisfied you are with how you're aging, the more likely you are to practice preventive health measures like eating right, exercising and quitting smoking.⁶⁵ You're also more likely to be able to live and function independently,⁶⁶ and have a much better shot at living longer (up to 7.5 years longer) than people who are dissatisfied with aging.⁶⁷ ◇



Hormone Therapy and Cognition

Both the KEEPS and TEAAM studies will examine the role of hormones in cognitive function (the ability to learn and remember information, organize, plan and problem solve). We won't know the results of these studies for years, however. So let's take a look at some results from other research in 2008:

- Researchers at the University of Connecticut in Farmington gave 57 healthy, postmenopausal women either an ultra-low dose of micronized estradiol (a form of estrogen) or a placebo (sugar pill) for three years. They found no change in the women's cognitive function or levels of depression, even when the results were evaluated based on age.⁵⁰
- Researchers in the Netherlands randomly assigned 237 healthy men between the ages of 60 and 80 to receive either supplemental testosterone or placebo twice a day for six months and measured their cognitive and physical function via a variety of tests. At the end of the trial, the men who received the testosterone showed increased lean body mass and less fat compared with placebo, but with no concurrent improvement in physical function. They also showed no change in cognitive function or bone density. While they showed improved sensitivity to insulin, levels of the "good" HDL cholesterol declined.⁵¹
- Researchers in Hong Kong found that the more "free" testosterone men had in their blood, the lower their risk of memory loss and Alzheimer's disease. However, they also found that total testosterone levels were not related to the risk of either condition. Total testosterone measures both free and bound.⁵² "Free" testosterone refers to circulating testosterone that is not bound to blood proteins (mainly sex hormone binding globulin). It is the free testosterone that is available to enter cells and cause hormonal effects, so this is a biologically active fraction.

The disparate results of these and other studies on the effects of hormones related to cognition are likely due to numerous factors, said Sanjay Asthana, MD, who is overseeing the cognitive function arm of KEEPS and who has completed extensive work on the role of hormones in cognition.

"Our belief is that the form of estrogen used in these trials is critical," he said. Most studies that showed little or no effect of supplemental estrogen on cognition used equine estrogen like that used in the WHI study. Yet most animal studies that show cognitive benefits from estrogen used estradiol, a form of estrogen identical to a woman's own. Laboratory studies show that estradiol enhances neurotransmitters (chemicals that enable communication between neurons in the brain); prolongs the life of brain cells; and enables brain cells to establish new synapses, or connections.

"Estradiol is several times more efficacious than equine estrogen," said Dr. Asthana, "and there are very few studies looking at the mechanisms of estradiol in terms of cognition."

Another factor is the composition of the progesterone used in trials with women who still have their uterus. As Dr. Manson noted on page 14, the type of progesterone used with estrogen likely affects health outcomes.

Finally, Dr. Asthana believes that how the estrogen is delivered is important. Estrogen delivered through a skin patch or cream provides more usable estrogen because, unlike oral forms, it doesn't go through the liver where it undergoes conversion to less potent forms and increases the amount of sex hormone binding globulin, which reduces its biological availability.

Given that one arm of KEEPS uses estradiol, micronized progesterone and a patch delivery system, it's possible that the results in terms of cognition may be quite different from those seen in the WHI and other previous studies. ◇

VITAMIN D: The Sunshine Hormone

You might call 2008 "The Year of Vitamin D." Although our understanding of the effect of the "sunshine vitamin" (which is really a hormone) on health and disease has been building for several years now, 2008 appeared to be a tipping point.

More than 2000 scientific articles and reviews on vitamin D were published last year. Low levels of vitamin D were linked to everything from an increased risk of Parkinson's disease, heart-disease and heart-related deaths, to stroke and even a higher risk of cesarean sections in pregnant women.^{53,54,55}

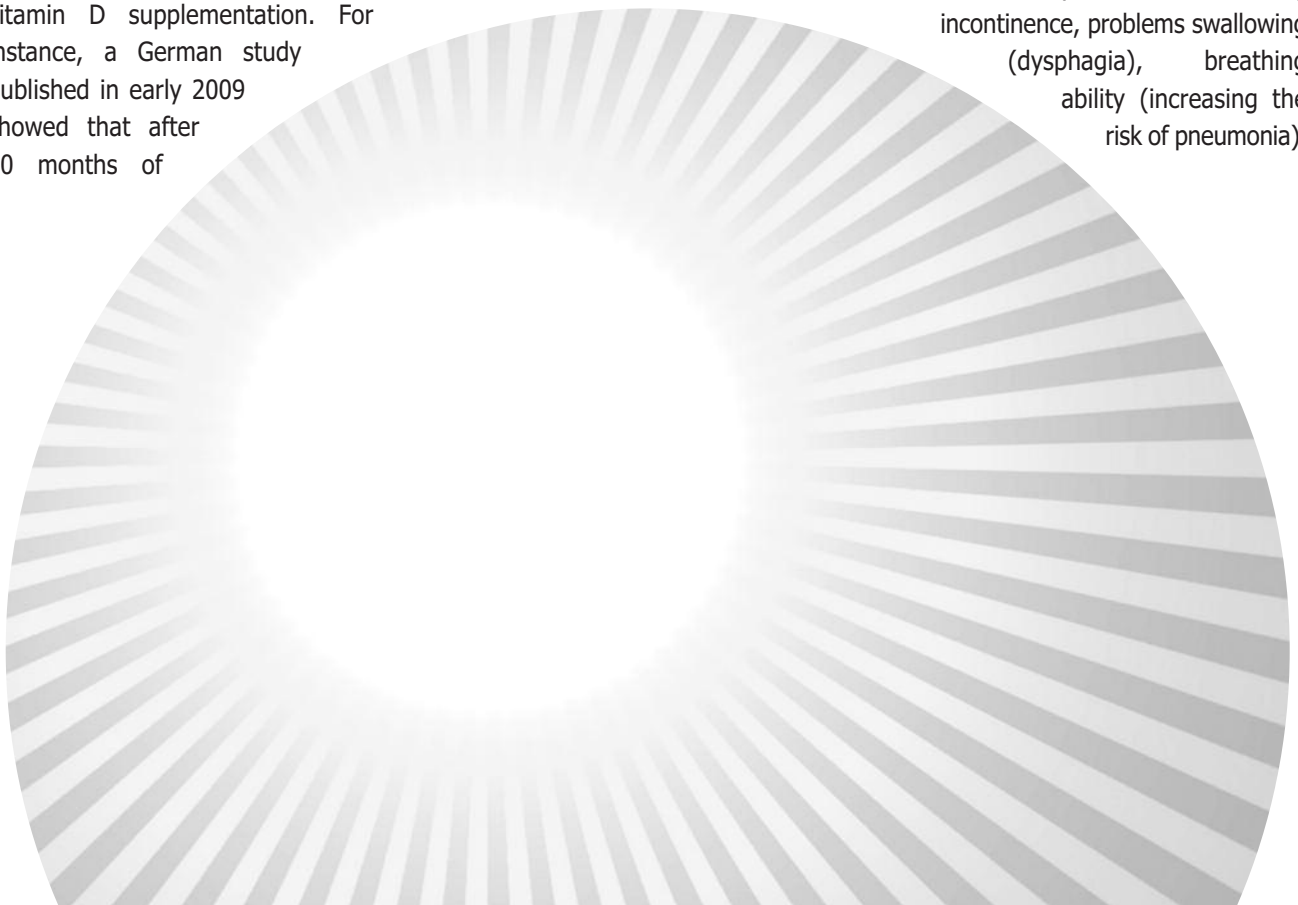
Meanwhile, tests ordered to determine vitamin D levels in patients soared 75 to 90 percent in the nation's largest diagnostic labs⁵⁶ and an article in *The New York Times* in February suggested the vitamin might be the "nutrient of the decade."⁵⁷

While studies linking vitamin D deficits and disease are as prevalent as popcorn at the movies, we're also starting to see results from studies examining the effect of vitamin D supplementation. For instance, a German study published in early 2009 showed that after 20 months of

supplementing with 1,000 mg of calcium and 800 IU of vitamin D, elderly men and women living on their own reduced their risk of falls 39 percent more than those who only supplemented with calcium. The supplementing group also had significantly greater muscle strength than the calcium-only group.⁵⁸

A small study examining the effects of vitamin D supplementation on blood pressure found that exposing 18 people with hypertension to ultraviolet B light 10 minutes at a time, three times a week for three months not only increased vitamin D levels about 180 percent, but brought their blood pressure down to normal.⁵⁹

The impact of vitamin D on age-related diseases appears enormous. We've known for a long time that vitamin D deficiency significantly increases the risk of osteoporosis and fracture, as well as the loss of muscle mass and strength, leading to frailty. Now, however, studies find that low vitamin D levels may contribute to urinary incontinence, problems swallowing (dysphagia), breathing ability (increasing the risk of pneumonia),



Vitamin D: The Sunshine Hormone *continued*

age-related macular degeneration, dementia, influenza and several cancers, including colon, breast and prostate.⁶⁰

Yet studies show that 40 to 100 percent of elderly men and women living in the community, and more than half of postmenopausal women taking osteoporosis medication, have clinically low levels of vitamin D.⁶²

Symptoms of vitamin D deficiency include muscle and joint aches, fatigue, low immunity, trouble sleeping, and mood changes, such as depression.

How Much?

Ideally, experts suggest children and adults maintain vitamin D blood levels of 30 ng/ml (nanograms per milliliter). Current recommendations call for dietary intake of 200 IU a day for adults to age 50, then 600 IU for those 71 and older.⁶¹ Most experts, however, say those recommendations are too low, and recommend 800 and 1,000 IU a day, higher if you take medications that can interfere with vitamin D absorption, have a deficiency or have dark skin. The panel that sets recommended dietary guidelines will likely recommend higher levels for adults in a couple of years.⁶²

The American Academy of Pediatrics doubled its recommendation for all children from 200 IU to 400 IU a day in 2008.⁶³

Getting enough vitamin D, however, is easier said than done. Our best source comes from sunlight, which our skin transforms into the vitamin. But from October through May, when the sun is farthest away from the earth, it's nearly impossible for people living in the northern hemisphere to get adequate vitamin D from sunlight alone. Plus, using sunscreen, covering your body with clothing, and even some medications can affect how much vitamin D your body can produce from sunlight.

There is some vitamin D in food—primarily fatty fish like salmon and tuna and egg yolks. Other foods are fortified with vitamin D, which include cereals, milk and orange juice.

Still, most experts say the best way to get enough vitamin D is with supplements.

Worried that you might be deficient in vitamin D? Talk to your healthcare professional about testing your blood levels. What you find might surprise you. ◊



WHAT WE'VE BEEN UP TO in 2008

Things have been busy at KLRI during the past year. You've already heard about our progress on KEEPS and TEAAM. Here is a snapshot of some of the other things we've been up to:

STATIN USE AND EXERCISE

The results of a KLRI study published in the *Journal of the American Aging Association* this summer showed that statin use in older adults does not negatively affect aerobic exercise or high-intensity weight training. Statins are the most frequently prescribed medication for high cholesterol levels. Because statins can deplete levels of an enzyme called Co-Q10, which plays a role in energy metabolism, there has been concern that it could interfere with individuals' ability to exercise. Indeed, people taking statins often complain of weakness and muscle tenderness while engaging in strenuous physical activity.

Yet none of the participants in this study had any issues with weakness or muscle tenderness while exercising.⁶⁸

The three-month study was conducted in 12 men and women ages 55 to 76 who had normal cholesterol levels and were not overweight. They also were not using any cholesterol-lowering medicines prior to the study. After baseline measurements of their oxygen, muscular strength and aerobic endurance, participants were each given 40 mg of statins for two weeks. The dosage was then increased to 80 mg for the remaining 10 weeks.

To measure their aerobic capacity, participants rode a stationary bike until they either achieved maximum oxygen intake or approached their maximum heart rate. To measure strength and muscle endurance, participants did upper and lower body presses, performing as many repetitions as possible. After 10 weeks, participants' LDL cholesterol had dropped an average of 51 percent and total cholesterol 60 percent with no significant changes in exercise parameters. The KLRI lead investigator, Dr. Tinna Traustadóttir, interprets these findings to mean that statins do not impair muscle function in most people. However, it is likely that



some fraction of people are vulnerable to adverse effects of statin on muscle, perhaps due to genetic variations. Identifying and studying statin effects on muscle function in this population and figuring out which genes cause the susceptible state is a task for future research.

OMEGA-3 FATTY ACIDS AND ENDOCRINE/ IMMUNE FUNCTION

Omega-3 fatty acids are valuable fats found in nuts, seeds and fatty fish like salmon and tuna. These “good” fats have been found to have significant anti-inflammatory and antioxidant properties. Supplementing with these fats can reduce the risk of certain heart disease, lower triglycerides, prevent or reduce depression, and relieve inflammatory pain.

These fats also make up cell membranes. Cell membranes become stiff and viscous with age, losing the fluidity that enables receptors on its surface to receive and send signals to other cells. These changes play a role in many age-related cognitive changes, from mild memory loss to dementia. Yet, people who consume a diet high in omega-3 fatty acids seem to be protected against this loss.

Could the omega-3 fatty acids provide some form of protection against these age-related membrane changes?

Possibly. In a study published in the journal *Hormonal and Metabolic Research* in March 2008, KLRI researchers reported that after 10 weeks on a diet high in omega-3 fatty acids, participants demonstrated significantly greater insulin sensitivity and lower levels of some circulating inflammatory markers. They also released fewer fat molecules that contribute to inflammation and oxidation.⁶⁹ What this all means remains to be seen; but, as you’ve learned throughout this State of the Science report, reducing oxidative stress and inflammation and improving insulin sensitivity are all pathways to reducing the risk of many age-related diseases.

Vinegar and Insulin Sensitivity

One in five adults over age 60 has diabetes, primarily type 2 diabetes, the type related to weight, lack of physical activity and poor diet. People with type 2 diabetes also risk early death, heart and kidney disease, nerve damage and a host of other complications that can make their later years miserable. An underlying pathology of diabetes is the insulin resistance discussed in more detail on page 7, which leads to high blood glucose levels. But guess what? That same household staple used to clean coffee pots, wash windows and create a salad dressing may also be able to lower glucose. Yes, “sour wine,” aka, vinegar, appears to have significant effects on blood glucose in both healthy people and those with diabetes.^{70,71,72,73}

The question is, just how does simple liquid like vinegar *do* this? Theories abound. Perhaps vinegar slows the rate at which food is digested, which helps moderate blood glucose levels. It might interfere with the digestion of complex carbohydrates, or spur the absorption of glucose already in the blood to the liver where it is converted into glycogen; it might also make you feel full faster and for longer, reducing the amount of calories you take in.

To find out, KLRI researchers have developed a small pilot study in which participants will receive either a placebo liquid or vinegar followed by a light meal. Researchers will then test for glucose absorption over a three-hour period while participants rate their hunger and fullness. Stay tuned!

As you can see, 2008 was a busy and productive year for biogerontologists throughout the country, as well as for KLRI scientists. We look forward to further developments and to keeping you updated on the most exciting findings in aging-related science.

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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.

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