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BioMarker Pharmaceuticals Develops Anti-Aging Therapy

By Saul Kent, Director,
Life Extension Foundation

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The scientific staff of BioMarker Pharmaceuticals (from left to right):Tomoshi Tsuchiya, M.D., Hyon-Jeen Kim, Ph.D., Joseph Dhahbi, M.D., Ph.D. (Senior Scientist), Xi Zhao-Wilson, Ph.D., MBA (President/CEO), Stephen R. Spindler, Ph.D. (Head of Technology), Patti Mote, BA, Glenn Shea, BS, Kyung-Youp Kim, B.S.

There is now scientific evidence that a therapy can slow the aging process in laboratory animals. BioMarker Pharmaceuticals (www.biomarkerinc.com), a new company funded by Life Extension, has discovered that metformin, a drug used to treat diabetes, can mimic many of the changes in gene expression found in calorically-restricted mice, which live much longer, healthier lives than normally-fed mice.

Caloric restriction (CR) is the most effective method of slowing aging, preventing diseases such as atherosclerosis, diabetes and cancer, and extending maximum life span in mammals. Since the 1930s, studies in rats, mice, monkeys and other species, have demonstrated that CR can maintain life, health and youth in animals for extended periods of time.

An Unprecedented Medical Breakthrough

The discovery that a clinically-used drug (metformin) produces genetic effects similar to those of caloric restriction, including life span extension, is an unprecedented breakthrough in medicine, with astonishing implications for us all. For thousands of years, the medical profession has strived to prevent and treat killer diseases, but has never been able to find a method of intervening in the aging process.

Advances in sanitation, nutrition and medicine have increased life expectancy to a considerable degree. In the year 1900, the average life span in the U.S. was only 47 years. By 2000, it had risen to the high 70s. This has led to increasing numbers of people reaching advanced ages, but has had no impact whatever on the maximum age reached by the oldest of the old. Today, it is still very rare for anyone to live beyond the age of 107, although increasing numbers of people reach 100. In ancient Greece, the average life span was in the early 20s, but the longest lived people still reached 100+.

In the U.S. today, the average 50-year-old woman survives to age 81. If we were to conquer the two leading causes of death—cancer and heart disease—it would only extend the woman's life span to 88, and these added years would be a time of declining health and vigor. If we were to slow aging, on the other hand, the woman would not only be less likely to die of cancer, heart disease and other killers, but would also be healthier, more vigorous and more youthful. She would live to well over 100, with the prospect of a much longer healthier life span from later advances in the control of aging. (Fig. 1)[1]

An authentic advance in aging control would, in just a few short years, produce more health and longevity benefits than have yet been produced in the history of the planet. It would break through the hitherto impenetrable barrier that has kept every person on Earth in chains since the beginning of time. Just as the ability to leave Earth's orbit gave us the possibility of reaching the moon, the planets, other star systems and galaxies, the ability to extend the healthy human life span would give us the possibility of living in good health well beyond the current maximum human life span.

BioMarker has taken the first revolutionary step in developing an authentic anti-aging therapy. Before going into the details of this advance, let's take a look at the ground-breaking technology used by BioMarker to progress so quickly.

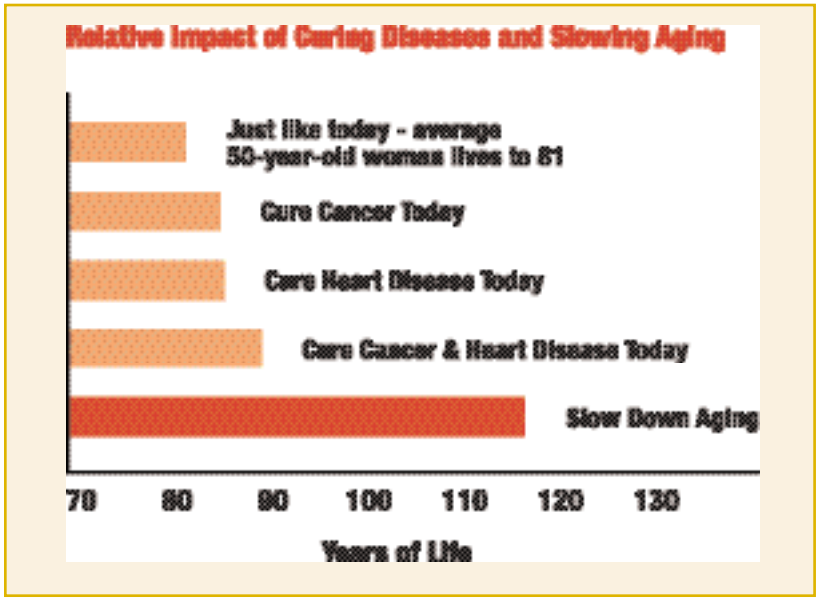

Fig. 1. A therapy to slow aging would add far more years of healthy, youthful life to the human life span than curing cancer and heart disease. (Adapted from Martin GM, LaMarco K, Strauss E, Kelner KL, Research on Aging: The End of the Beginning, *Science*, 299:1341, 28 Feb. 2003.)

Measuring The Rate Of Aging

Diseases can be diagnosed and, in many cases, treated effectively because they afflict a subset of the population and have known causes, biomarkers, risk factors and characteristics. Influenza, pneumonia and AIDS, for example, are caused by pathogens that produce symptoms such as respiratory distress, aches and pains and immune dysfunction. Cancer is characterized by the accelerated proliferation of aberrant cells; heart disease and stroke by atherosclerotic plaques and abnormal blood clots; and diabetes by elevated blood levels of glucose and insulin.

Aging, on the other hand, affects everyone, is characterized by the deterioration of every tissue, organ and system in the body, and occurs in a progressive manner throughout most of the life span. The consequences of aging are so widespread, pervasive and intertwined with degenerative diseases that it is very difficult to measure the rate of aging. Since many of the features of aging are also characteristic of crippling and killing diseases—of which aging is a major, underlying cause—it is difficult to separate aging from disease processes.

The one biomarker that has been used effectively as a measure of aging is maximum life span. Every species has a characteristic life span that is determined by its rate of aging. The maximum life span of mice and rats is three years, the maximum life span of chimpanzees is 50 years, while the maximum life span of human beings is 100+.

The ability of caloric restriction to extend the maximum life span of mice and rats to as long as four-to-five years is thus strong evidence that CR retards their rate of aging.[2] Further evidence of CR's ability to slow aging and extend youth is its ability to prevent the immune dysfunctions of old age, improve DNA repair, reduce damaging free radical activity, lower glucose and insulin levels, maintain fertility at advanced ages, boost energy levels, increase protein synthesis, reduce the accumulation of damaged proteins, inhibit the inflammatory responses of aging, lower blood levels of cholesterol and triglycerides, counteract neurodegeneration and prevent the age-related decline in the health-building hormone DHEA (dehydroepiandrosterone). CR also prevents,

postpones the incidence of and reduces the severity of the diseases that normally kill mice and rats, such as cancer, kidney disease and cardiovascular disease.[3]

At advanced ages, calorically restricted animals are thinner, smaller and more youthful looking than normally-fed animals. (Fig. 2) The most striking difference between CR and normally-fed animals, however, is their activity level. Thirty-month-old normally-fed mice, which are roughly equivalent to 75-year-old humans, are sluggish, bloated, sedentary and often have malignant tumors, if they are still alive. In contrast, 30-month-old CR mice exercise vigorously, often in acrobatic fashion, and are highly inquisitive. In observing both groups of "elderly" mice, it is obvious that the CR animals are younger and healthier than normally-aging mice. At the end of this article, readers are given contact information to enable them to learn more about BioMarker Pharmaceuticals. Among the items available is a motion picture sequence showing how much more active and youthful CR mice are than normal mice, at advanced ages.

Since CR can prevent the diseases of aging, maintain health and youth in animals at advanced ages, and extend maximum life span (in mice and rats) up to the equivalent of 160 years in humans, it is now accepted by scientists as an authentic way of slowing aging in mammals. Ongoing CR studies at the National Institute on Aging (NIA) and the University of Wisconsin have shown that CR produces similar anti-aging and anti-disease effects in rhesus monkeys as in rodents, although it is too early to tell if the maximum life span of CR monkeys will be extended.[4] Preliminary data from the NIA study indicate that CR is reducing cardiovascular disease, diabetes, cancer and liver failure in rhesus monkeys.[5]

During a stay in Biosphere II in Arizona, the eight inhabitants were unable to grow enough food for them to eat a normal diet. As a result, they experienced unintentional caloric restriction for almost two years. One of the Biosphere II crew, Dr. Roy Walford of UCLA Medical Center, made regular biochemical and physiologic measurements of the crew, which showed strikingly similar anti-aging effects to those found in mice, rats and monkeys.[6]

The inhabitants of Okinawa consume a healthy diet that is 20% lower in calories than those in the rest of Japan. A study found that—in the 1970s in Okinawa—death rates from stroke were reduced by 41%, cancer by 31% and heart disease by 41% below the rest of Japan. The mortality rate for 60 to 64 year olds living on Okinawa was half the rate found elsewhere in Japan, and the incidence of centenarians (people living to 100+) on the island was 2 to 40 times greater than in other Japanese communities.[7]

When George Roth and associates at the National Institute on Aging analyzed data from the Baltimore Longitudinal Study of Aging, they found that survival was greater in men who had lower body temperature, lower plasma insulin levels and higher DHEA levels than others in the study.[8] Since CR lowers body temperature and plasma insulin levels in both rodents and monkeys and increases DHEA levels in monkeys, these findings in the healthier men in the Baltimore study suggest that a common mechanism affects these changes in both non-human and human primates. Unfortunately, information is not available on the caloric intake of the subjects in the Baltimore Longitudinal Study, but since the men were not intentionally put on a CR diet, it is likely that anti-aging therapies that have the beneficial effects of CR can be developed. This is BioMarker's goal.

Fig. 2. The mouse on the left has been on a caloric restriction diet. The CR mouse is lighter, healthier and younger than his normally fed counterpart. Both mice are 20 months of age.

A Drawback Of Maximum Life Span Studies

A major drawback in using maximum life span to measure the rate of aging is that life span experiments take too long. Monkeys and humans live for such long periods of time that any attempt to determine whether a therapy can extend their maximum life span takes decades. The NIA and Wisconsin CR studies in rhesus monkeys have been going on for 10 to 15 years, yet it is still too early to determine whether CR is extending mean life span in these animals, and we won't discover the effect of CR on their maximum life span for another 30 to 40 years. No one has ever attempted a study to see if maximum life span can be extended in humans because such a study would likely outlast the life span of the researchers. Even in relatively short-lived mammals such as mice and rats, a study of maximum life span can take four to five years. Moreover, these studies are not only time-consuming, but costly.

Because of the time and money needed for maximum life span studies, scientists have been searching for short-term assays (biomarkers of aging) to measure the rate of aging in humans. A true biomarker of aging would measure a fundamental biologic function that occurs in everyone throughout the life span. It would have to be a function that underlies the diseases of aging, but is not a disease process itself, and that cannot be reversed by therapies that prevent or treat diseases. For example, a sedentary man of 50 with elevated cholesterol and blood pressure has a higher-than-normal risk of heart attack and stroke. If the man starts to take anti-cholesterol and anti-hypertension medications and exercises regularly, he will have lower cholesterol and blood pressure readings at age 60, but still be biologically older at 60 than he was at 50.

BIOMARKER'S SCIENTIFIC STAFF

CHAIRMAN OF SCIENTIFIC ADVISORY BOARD:

Stephen R. Spindler, Ph.D., Prof. of Biochemistry, Univ. of California, Riverside Dr. Spindler is one of the pioneers in applying high-density microarray technology (gene chips) to the measurement of gene expression of thousands of genes in normally aging, calorically-restricted, and long-lived dwarf mice. He has published 49 scientific papers in peer-reviewed journals in the fields of gerontology, molecular biology and endocrinology.

PROJECT DIRECTOR, SENIOR SCIENTIST:

Joseph Dhahbi, M.D., Ph.D. Dr. Dhahbi has conducted and supervised much of BioMarker's research. He has published 12 scientific papers in peer-reviewed journals in the fields of gerontology and molecular biology.

OTHER SCIENTIFIC PERSONNEL:

Shelley Cao, Ph.D., Hyon-Jeen Kim, Ph.D., Tomoshi Tsuchiya, M.D. Patricia L. Mote and Glen Shea.

SCIENTIFIC COLLABORATORS:

1. George S. Roth, Ph.D., Senior Guest Scientist, Nutritional & Molecular Physiology Section, National Institute on Aging, NIH, Baltimore, MD.
2. Donald K. Ingram, Ph.D., Chief, Behavioral Neuroscience Section, Gerontology Research Center, National Institute on Aging, NIH, Baltimore, MD.
3. Mark A. Lane, Ph.D., Project Manager, Merck & Company, Rahway, NJ.

Drs. Roth, Ingram and Lane have been conducting a long-term study of caloric restriction in monkeys at the National Institute on Aging. They have also conducted research aimed at finding CR mimetics. They are collaborating with BioMarker to conduct gene chip studies on the tissues of normally-fed and CR monkeys.

4. James Nelson, Ph.D., Dept. of Physiology, Univ. of Texas, Dallas.
5. Brian Allan, Ph.D., Dept. of Physiology, Univ. of Texas, Dallas.

Drs. Nelson and Allan have contributed to BioMarker's microarray studies in monkey tissues.

6. Andrzej Bartke, Ph.D., Dept. of Physiology, Southern Illinois University, Springfield, IL.
7. Richard A. Miller, M.D., Ph.D., Prof. of Pathology, Univ. of Michigan, Ann Arbor.

Drs. Bartke and Miller are collaborating with BioMarker to test and analyze the tissues of long-lived dwarf mice with gene chips.

8. Gerold Grodsky, Ph.D., Prof. of Biochemistry, Biophysics and Medicine, Univ. of California, San Francisco and Founding Advisory Editor, Diabetes Technology and Therapeutics.

Dr. Grodsky is an authority on diabetes research, treatment and management. He is advising BioMarker on the conduction of studies aimed at finding new anti-aging, anti-disease therapies.

9. Michael West, Ph.D., President and CEO of Advanced Cell Technology, Worcester, MA.

Dr. West is advising BioMarker on progress in the development of therapeutic stem-cell technologies and is expected to collaborate on studies testing stem cell therapies with BioMarker's technology.

Another requirement for a true biomarker of aging is that it has to change fast enough to be able to see significant differences over relatively short periods of time. If a function changes so slowly that it takes 10 years or more before the change is significant, it would be impractical to use it as an assay of aging.

Finally, a biomarker of aging should be relatively non-invasive, such as a blood test or skin biopsy. A heavily invasive test, such as a brain biopsy, would be costly, time-consuming and would represent an unacceptable risk for most people. Testing for biomarkers of aging will have to be done at least once a year to generate convincing scientific evidence of the anti-aging effects of therapies. The results of such tests will need to provide scientific data quickly in order to screen therapies for their ability to retard or reverse aging.

Gene Expression: True Biomarkers Of Aging

Since genes control every aspect of biologic life, including health, aging and longevity, and caloric restriction extends healthy life span, a logical approach in the search for true biomarkers of aging is to compare gene expression in normally aging animals with gene expression in CR animals. In the 1980s, a team led by Arlan Richardson (then at Illinois State University) began to explore this comparison in rats, using tools of molecular biology, such as Northern Blot analysis. They looked at gene expression in the liver cells of 18-month-old, normally-fed rats and 18-month-old CR rats and found that protein synthesis, mRNA levels and nuclear transcription were increased two- to three-fold by caloric restriction.[9]

The problem with using these tools of molecular biology to search for biomarkers of aging, however, is that such experiments are slow, laborious and expensive. Those problems have been solved by a new technology—high-density DNA microarrays (gene chips)—developed by companies such as Affymetrix in Santa Clara, California. Gene chips have enabled scientists to detect gene expression quickly in thousands of genes at a time.

Richard Weindruch and Tomas Prolla of the University of Wisconsin have used gene chips in aging research. In 1999, they used Affymetrix gene chips to measure the

BIOMARKER'S MANAGEMENT

PRESIDENT/CHIEF EXECUTIVE OFFICE (CEO): Xi Zhao-Wilson, Ph.D., MBA.

Dr. Zhao-Wilson has more than 15 years experience as a senior executive of start-up, early stage and established companies in the biotech industry. She was corporate development executive at Aviron, a biopharmaceutical company that recently merged with MedImmune. She was a founder and CEO of InCell, a biomedical company, and served as board director and senior executive officer at Baekon, a biotech company. Prior to her corporate career, Dr. Zhao-Wilson was a research scientist at Stanford Medical Center, where she conducted research in the field of gene regulation of cancers related to steroid hormone receptors. Dr. Zhao-Wilson is a professor at the Chinese Academy of Preventive Medicine. She received her MS in molecular genetics and cell biology from the Chinese Academy of Sciences, her Ph.D. in cellular and developmental biology from the Ohio State University and her MBA from the Fisher School of Business.

CHIEF FINANCIAL OFFICER (CFO): Matthew J. Franklin, MBA. Mr. Franklin has held a broad range of positions for several early stage companies. He held the top financial position for ACLARA BioSciences, a public "lab-on-chip" technology company, which had one of the most successful IPO's in biotech history. At ACLARA, he oversaw finance, accounting, administration, HR, facilities and IT. He assisted in raising over $40 million dollars in venture capital, corporate partnerships, government grants and debt financing. Prior to ACLARA, he was co-founder and CFO of BioLumin Corp., a medical device company. Dr. Franklin also served as CFO of PeopleTrends (Internet) and AF_X, a medical device company. Mr. Franklin received a BS in business from the Univ. of Southern California and an MBA from Santa Clara University.

VICE PRESIDENT, BUSINESS DEVELOPMENT: Paul C. Watkins has over 20 years experience in business development and R&D with biotechnology, biomedical, genomics, life science and pharmaceutical companies, ranging from start-ups to the post-IPO stage. He has significant international business experience in China, Japan and Europe. Prior to BioMarker, he was Senior Director, Scientific Affairs and Business Development for Ancile Pharmaceuticals. He also was a senior business development executive at Sequana Therapeutics, a pioneer in commercializing DNA technology for drug discovery, and at Axys Pharmaceuticals, Sequana's acquirer (Axys has since been acquired by Celera Genomics). At Life Technologies, he managed molecular biology R&D projects. Mr. Watkins earned his MS in animal cell science, molecular biology and genetics at the Massachusetts Institute of Technology (MIT) and his BS in microbiology and public health from Michigan State University.

BIOMARKER'S BOARD OF DIRECTORS

CHAIRMAN OF THE BOARD: Saul Kent has played a major role in efforts to extend the healthy human life span for 39 years. He is a founder (in 1980) and director of the Life Extension Foundation in Hollywood, Florida, a non-profit organization that disseminates life-saving information and funds pathbreaking research. In addition to being a founder of BioMarker Pharmaceuticals, Kent has founded two companies in Rancho Cucamonga, California: 21st Century Medicine, which engages in research to cryopreserve organs for transplant; and Critical Care Research, which is developing a portable, automated hypothermia system for clinical medicine and advanced methods of resuscitation. Kent has also overseen research projects funded by Life Extension, including studies to determine if dietary supplements and drugs can extend life span in animals, studies aimed at rejuvenation of the aged, transgenic mouse studies to elucidate mechanisms of aging, and the exploration of methods to rejuvenate the immune system.

BOARD MEMBER: Xi Zhao-Wilson, Ph.D., MBA – See description of Dr. Zhao-Wilson's qualifications on the left.

BOARD MEMBER: Michael West, Ph.D. is President/CEO of Advanced Cell Technology in Worcester, Massachusetts, a leader in cloning research that is seeking to develop anti-aging and anti-disease therapies from cloned embryonic stem cells. Dr. West was founder of Geron Corp. in Menlo Park, California (now a public company), which has pioneered in telomerase and stem cell research. Before that, Dr. West conducted breakthrough research in cell biology at the University of Texas in Dallas.

BOARD MEMBER: Victor V. Vurpillat, Ph.D. has helped fund 14 start-up companies. For 15 years, Dr. Vurpillat was Vice-President of Safeguard Scientific, a broad-based, high-tech company that provides financial and management services for its associated companies, which have included Novell Data Systems, Compucom, Coherent Systems, Cambridge Technologies and QVC. Among the other companies, Dr. Vurpillat either founded or worked for are: InCell, a biotech company, where he was Chairman of the Board; Triquest Ventures, a venture capital firm; SpanWorks, a joint venture with Toshiba of Japan; and Telerate, a worldwide financial information provider, which was purchased in 1987 by Dow Jones for $1.7 billion. Dr. Vurpillat's companies have generated more than $10 billion in stock market valuation. He holds a degree in Mathematics from California Polytechnic University, an MBA from Pepperdine University, and a Ph.D. in Human Behavior from Newport University.

expression of 6,347 genes in the gastrocnemius (leg) muscles of young (5-month) and old (30-month) mice. When they compared gene expression in normally aging mice with gene expression in CR mice, they found that many of the genetic changes of aging were reversed in the CR animals.[10] Since then Weindruch and Prolla have conducted similar experiments in the brain and heart of mice and monkeys.

BioMarker scientists made a major breakthrough by determining that 70% of the age-related changes in gene expression reversed by long-term CR (over two years) are reversed in only two to four weeks after mice are placed on a CR diet. BioMarker used Affymetrix gene chips that measure changes in 12,000 genes in these studies. This discovery established the scientific foundation for the company's proprietary technology platform.

Screening For Anti-Aging Therapies

Comparing the expression of thousands of genes at a time makes it possible for scientists to develop genetic profiles of aging in different organs in mice, monkeys and humans, and to discover what age-related genetic changes are prevented or reversed in models of life extension such

as caloric restriction. When the key genes that govern aging are identified, scientists will be able to target the genes, the proteins they produce and the biologic mechanisms they affect in order to develop new drugs and other therapies to slow aging, prevent diseases and extend healthy life span.

Gene-chip technology has enabled scientists to measure the expression of thousands of genes at a time. Weindruch and Prolla demonstrated that you can screen for anti-aging therapies over a 25-month period. BioMarker Pharmaceuticals has made major strides in demonstrating that the screening process can take place over a much shorter period and that the benefits of CR can occur at advanced ages.

The company has also found evidence for genetic mechanisms that may be involved in the prevention of cancer and a drug (metformin) that may be an authentic anti-aging therapy.

How CR Affects Life Span Late In Life

Most scientists assume that the anti-aging, life span extending effects of CR involve the gradual prevention of age-associated genetic and biologic changes, and that these effects lessen with advancing age. The early studies found that, while CR could extend life span dramatically when started early in life, it shortened life span when started later in life. Then, Weindruch and Walford found that CR can extend the life span of middle-aged (one-year-old) mice if their diet is restricted gradually to allow them to adapt to reduced caloric intake.[11]

BioMarker has found that CR can alter gene expression in male, long-lived hybrid B6C3F1 mice very rapidly in both young and old animals. Within four weeks, short-term CR reversed 70% of the age-related changes in gene expression reversed in mice undergoing CR for two years or more. Short-term CR induced a genetic expression profile associated with health and longevity. In some cases, gene expression was elevated, in other cases reduced, when compared to the gene changes found in naturally aging mice.

Furthermore, the company has found that CR is just as effective in extending life span late in life as it is early in life. In the BioMarker study, late-life CR increased the mean and maximum life span of mice by approximately 40% (Fig. 3) and slowed the onset of malignant tumors.[12] These findings are evidence that CR rapidly decelerates aging, even when started late in life. They also show that the rapid effects of CR on gene expression occur in the same time frame as the rapid effects of CR on longevity. This links the gene expression and longevity effects in a probable cause-and-effect relationship.

Types Of Genes Changed By CR

BioMarker's strategy is to search for the genes most critically involved in the aging process by exploring the changes in gene expression caused by caloric restriction in various tissues (liver, heart, brain and muscle) in mice, monkeys and humans throughout their life span, and to discover what gene changes occur when CR is started at different ages, and carried on for varying periods of time. BioMarker scientists are looking for changes in key regulatory genes that trigger secondary gene changes. They are looking for changes that occur in multiple tissue types very early in the crossover from the aging gene expression profile to the anti-aging gene expression profile.

The changes in gene expression caused by caloric restriction were separated into defined functional classes for further study. These include genes involved in carbohydrate, fat and protein metabolism; signal transduction for cell growth and proliferation, cell suicide (apoptosis); and the production of insulin, growth hormone and insulin-like growth factor-1; cellular protection against oxidative free radicals and other types of stress inducers; xenobiotic metabolism involved in detoxification of chemicals; and inflammation.

Among the metabolic genes that changed with CR were genes that indicate CR enhances the breakdown and turnover of whole-body protein. These changes should drive protein renewal throughout the body. Some of the genes reduced the enzymatic capacity for lipid biosynthesis and metabolism, which may account for the decreases in serum triglyceride and cholesterol levels in CR animals.[13]

Anti-Cancer Mechanisms Induced By CR

BioMarker has found changes in gene expression in CR mice that suggest several anti-cancer mechanisms induced by CR. One is the reduction of endoplasmic reticulum chaperone gene levels. Most proteins require the action of chaperone genes for biosynthesis, maturation, processing, transport and degradation.[14] Chaperones are

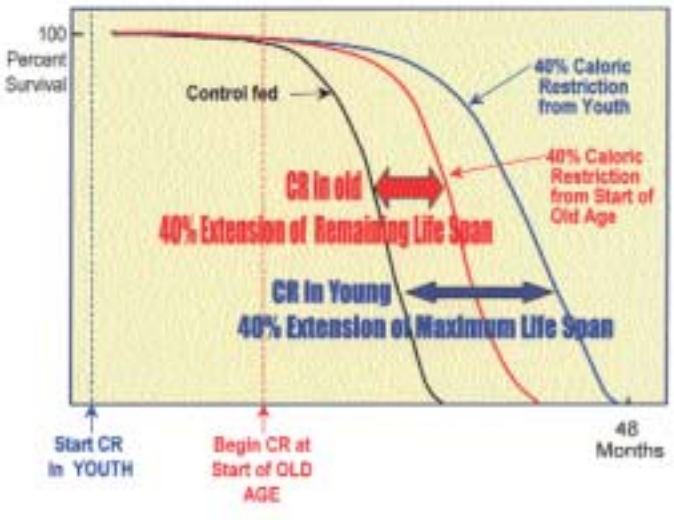

Fig. 3. Representation of the results of longevity studies conducted by BioMarker. The black line shows the life span of control mice who consumed a normal number of calories. The red line shows the life span of mice subjected to caloric restriction starting in old age. The blue line shows the life span of mice subjected to caloric restriction, starting very early in life. A major finding is that CR extended the remaining life span by the same proportion in both experimental groups.

Is Growth Hormone An Anti-Aging Or Pro-Aging Therapy?

Growth hormone (GH) and growth-hormone-releasing agents have been actively promoted as anti-aging therapies. Release of GH from the pituitary declines with advancing age[43] and reduced levels of GH (and IGF-1) almost certainly contribute to age-related loss of muscle mass, increase of body fat, loss of bone minerals[44] and impairment of cognitive function.[45]

These declines in function can be reduced or reversed by GH therapy.[44] The use of GH in anti-aging medicine is further supported by evidence that, in GH-deficient individuals, treatment with GH has a positive effect on several measures of psychological well-being and the quality of life.[46]

On the other hand, there is considerable evidence that excess GH can contribute to age-related diseases and shorten life span, and that life span-extending models such as caloric restriction (CR) and dwarfism triggered by gene mutation cause a decline in GH levels.[47] These findings suggest that GH therapy might have pro-aging effects.

The apparently opposite effects of GH on aging and diseases are not necessarily conflicting because they refer to different actions of GH and to different stages of life. The anti-aging actions of GH refer to its effects on body composition and function in elderly persons, rather than its role in determining life expectancy.[32] The anti-aging effects of GH therapy are typically observed after short-term treatment in GH-deficient subjects, however there is evidence that chronically elevated GH levels increase the incidence of cancer, cardiovascular disease, diabetes and kidney disease.[47]

It may be that short-term administration of GH in aging GH-deficient patients can effectively rejuvenate them, while GH given to persons with normal or above-normal GH levels has negative effects, including, perhaps, shortened life span. It's also possible that restoring other age-related hormone deficiencies in addition to GH can produce beneficial effects on health and longevity, while long-term administration of GH alone has deleterious effects. Clearly, further research is needed. BioMarker Pharmaceuticals will be exploring these critical issues by assessing the effects on gene expression in animals and humans receiving growth hormone (and other hormone) therapies.

involved in the building (folding) and repair (refolding) of proteins, which helps to maintain the integrity of cells that counter the damage caused by heat, oxidative, ischemic and inflammatory stress.

However, elevated chaperone expression, which occurs during aging, decreases apoptosis, a key mechanism that causes aberrant cells to self-destruct (commit suicide). There is evidence that chaperones increase the secretion of proteins that inhibit apoptosis and reduce apoptotic responsiveness to stress.[15,16] Apoptosis plays an important role in getting rid of cancer cells and other aberrant cells, including cells injured during the aging process. In every tissue, a balance must be struck between the need to maintain cell number and function, and the need to eliminate damaged, potentially toxic cells, including cancer cells. CR reduces chaperone levels in mouse liver, which increases apoptosis. This may be one of the reasons CR prevents cancer in organs such as the liver, which feature dividing cells.[17] In contrast, in non-dividing cells, such as neurons, CR appears to induce chaperone expression, which enhances cell survival[18] and may delay the onset of neurologic disorders of aging, including Alzheimer's disease, Parkinson's disease and stroke.[19]

Other Anti-Disease Mechanisms Induced By CR

Another mechanism linked to cancer and other diseases associated with aging is inflammation. Researchers have found changes in gene expression linked to the development of age-related pathologies in the liver, muscle and brain.[12, 21, 22] BioMarker's gene chip studies in mouse liver have found that aging is associated with other gene expression changes consistent with disease processes. The company found several gene expression changes involved in inflammation, which increases with advancing age, and has been associated with the development of several chronic diseases. BioMarker found that caloric restriction suppressed the age-related increase in inflammatory and stress response genes, which may be another reason for the ability of CR to prevent cancer and diminish the severity of autoimmune and inflammatory diseases in mice.

The liver is one of the primary organs involved in metabolizing, detoxifying and excreting potentially damaging chemicals and drugs. A decline in these functions in the liver has been found in aging mice, rats, monkeys and humans.[23] For example, cytochrome P450 activity, which plays a major role in the detoxification process, decreases 30% in humans after 70 years of age.[24] In rodents, there is compelling evidence for a decline in similar critical enzyme activities.[25]

In the BioMarker studies, aging decreased the expression of xenobiotic metabolism genes. This finding is likely responsible in part for the age-related decline in the metabolizing capacity of the liver, which is a recognized source of adverse drug reactions in aging mammals and may contribute to the increase in cancer with age in mice. Both short-term and long-term caloric restriction reversed the decrease in the expression of these genes, which may be another reason for the anti-cancer and anti-aging effects of CR.

The liver plays a major role in maintaining glucose (blood sugar) homeostasis, which is controlled by hormones such as insulin, glucagon, growth hormone (GH) and insulin-like growth factor-1 (IGF-1). Elevated levels of glucose and insulin are implicated in many age-related diseases,[26] such as type 2 diabetes, hypertension, heart disease and stroke, and are a hallmark of mammalian aging.

Caloric restriction reduces blood glucose and insulin levels in rodents, monkeys and humans.[6, 27, 28] Diseases associated with elevated glucose are reduced or eliminated entirely by CR. BioMarker's gene chip studies showed that the expression of genes associated with glucose and insulin were shifted to a more youthful profile. This result is consistent with the finding that CR mice are four times more insulin sensitive than normally-fed mice.[29]

The Most Valuable Breakthrough

The most valuable breakthrough made by BioMarker is the finding that a majority of the changes in gene expression caused by long-term CR (over two years) occur (in the liver) in only two to four weeks after placing mice on a CR diet. Traditionally, scientists have regarded CR as a slow process that incrementally prevents the accumulation of deleterious age-related changes in physiology and gene expression.[10]

BioMarker's finding that short-term CR rapidly shifts the gene expression profile of mice toward that of long-term CR is a profound paradigm changer. It introduces for the first time the idea that drugs can be rapidly assayed for their ability to mimic the anti-aging, anti-disease, life span-extending effects of long-term CR. Previously, it was thought that the drugs would have to be administered over an animal's lifetime to produce evidence that they can slow or reverse aging. Now, with the use of BioMarker's proprietary technology, it is possible to screen for anti-aging drugs in a matter of weeks.

BioMarker's use of high-density microarrays, which measure the expression levels of thousands of genes at a time makes it possible to conduct the screening process for anti-aging drugs **25 times faster** than any other technique currently available. The ability to measure the expression of thousands of genes at a time generates a huge amount of data per experiment.

This makes it possible to obtain enormous insights into aging and longevity with a few mice per drug in less than a month.

Rejuvenating The Elderly

BioMarker's finding that CR can produce anti-aging, anti-disease and life span-extending effects in old mice just as it can in young mice means that anti-aging drugs discovered with its technology should be effective in old people as well as young and middle-aged people. It gives the elderly hope for a longer, healthier life span, even if they are highly advanced in age.

BioMarker's research suggests it is possible that the ability of CR to extend life span in old animals occurs because it may be able to **reverse aging** and **rejuvenate the elderly**, not just slow down the aging process. If this is so, then drugs that mimic the effects of CR should be able to achieve both these objectives. This is of critical importance not just for the elderly, but for anyone over age 30, who has already begun to experience the degenerative effects of aging.

Quick Screening For Anti-Aging Drugs

The ability to screen for anti-aging drugs quickly and inexpensively is an enormous breakthrough. Pharmaceutical companies spend billions of dollars in their efforts to improve upon the largely inadequate therapies currently available for cancer, heart disease, stroke, Alzheimer's disease and other killers. They usually have to conduct lengthy, highly expensive studies on thousands of drugs in order to find even one promising new drug candidate.

BioMarker can save these companies huge amounts of money and time with its unique technology. The company can help them discover new drugs to combat aging and degenerative disease, as well as new uses for already approved drugs. BioMarker has been in contact with several large pharmaceutical companies, which have expressed strong interest in forging strategic alliances with the company.

BioMarker has also embarked on its own research program to search for anti-aging drugs. The power of the company's revolutionary technology has already been demonstrated in its first series of experiments. Five drugs were evaluated for their ability to mimic the gene expression profile of CR in the liver of mice using Affymetrix gene chips. Four of the compounds tested were glucoregulatory agents that produce a marked reduction in blood glucose and insulin and enhance insulin sensitivity in tissues, as CR does.[30] The fifth agent tested was a cancer chemopreventive agent.

The effects of the drugs were tested by feeding them to elderly mice as part of their diet. A normal control group and short-term CR and long-term CR groups were also included in the study. After a few months, the gene expression profiles of 12,422 genes were determined. The control group and the mice on long-term CR and short-term CR were compared to the drug-treated groups to determine the extent to which the drugs reproduced the effects of CR. Gene expression changes were verified by quantitative PCR and Northern Blot analysis.

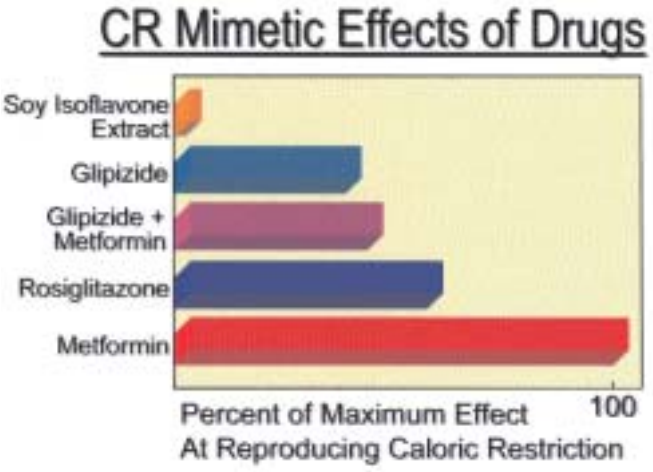

Fig. 4. The ability of drugs to mimic the gene expression effects of caloric restriction. BioMarker scientists found that metformin was most effective at reproducing the gene expression biomarkers of CR. Metformin's ability to do so is represented at 100% by the lowest bar in the chart, with the other drugs represented according to their lesser ability to mimic CR relative to metformin.

Metformin Stands Out From The Pack

The BioMarker scientists found that all the glucoregulatory agents reproduced some of the gene expression effects of CR, but that metformin was the undisputed star of the group, being twice as effective as the others in reproducing the effects of CR. The chemopreventive agent reproduced almost none of the effects of CR. Fig. 4 summarizes the gene expression changes. The overlap between the drug-induced gene expression profiles and those of CR is represented by the length of the colored bars.

The genes that were altered in expression by both metformin and CR are linked to drug metabolism and detoxification; energy metabolism; protein biosynthesis and degradation; cell growth and proliferation and the cytoskeleton. These findings suggest that metformin has more beneficial effects than the reduction of blood glucose and insulin, and that it may be an authentic anti-aging therapy.

Metformin Extends Life Span

Further evidence that metformin is an anti-aging therapy is a study conducted by scientists at the National Institute on Aging (NIA) showing that metformin extended the life span of mice by 20%. The results of this study were presented in November 2002 at an NIA-sponsored meeting at the Mayan Ranch in Bandera, Texas. BioMarker is now conducting a life span study with metformin to see if the company can reproduce these results.

In the late 1970s, Dilman and Anisimov at the N.N. Petrov Research Institute of Oncology in Leningrad (now St. Petersburg), Russia found that the life-long administration (2 mg/day) of phenformin—a glucoregulatory drug similar to metformin that causes more side effects—increased the life span of female C3H/Sn mice by 23%, while reducing the incidence of mammary tumors and other cancers in these animals.[31] The scientists proposed that phenformin delays the aging process, and that its effects may be similar to those caused by caloric restriction.

Finding Key Anti-Aging Gene Changes

One of BioMarker's major challenges is to find which gene expression changes are the keys to the anti-aging, life span-extending effects of CR. The use of microarrays has given BioMarker a powerful tool to map the gene changes involved in extending life span, but further research is needed to pinpoint which gene changes are critical in the process. BioMarker scientists believe that there may be a few regulatory gene changes that trigger most of the other gene changes that occur after animals are placed on CR, and that these changes may be among the earliest changes caused by CR. The company is conducting research to find these key gene changes.

Project Director, Senior Scientist Joseph Dhahbi, M.D., Ph.D. (left) and Stephen R. Spindler, Ph.D., Head of Technology of BioMarker discuss recent findings by the company.

There is now significant scientific evidence that aging can be retarded and life span extended by manipulating a small number of genes. Studies in yeast, worms and flies have shown that silencing or overexpressing certain genes can double or even triple the maximum life span of these forms of life.[32]

A Single Gene Change Extends Life Span In Mice

Since the mid 1990s, studies have generated evidence that **a single gene change** can radically extend the life span of mice. First, it was shown by Andrzej Bartke of Southern Illinois University and others that adult Ames dwarf mice (which are one-third the weight of normal Ames mice) live much longer than their normal counterparts.[32, 33] Then a comparable extension in life span was detected in Snell dwarf mice by Kevin Flurkey of The Jackson Laboratory in Bar Harbor, Maine and Richard A. Miller of the University of Michigan in Ann Arbor.[34, 35] **The life spans of these mice were extended by 40% to 70%!**

These mouse models have a single mutation—at the *Prop-1* locus in Ames dwarf mice and the *Pit-1* locus in Snell dwarf mice—which interferes with anterior pituitary development, which causes deficiencies in growth hormone (GH), prolactin (PRL) and thyroid-stimulating hormone (TSH).[36] It has been found that the deficiency of PRL is probably not involved in extending the life span of these

mice, that the deficiency of TSH may be involved to a small degree, but that the deficiency of GH (and insulin-like growth factor-1, which is made in response to GH) may be a primary reason these mice live so much longer. One finding that suggests this is that mice who cannot respond to growth hormone because their GH receptor gene has been knocked out, live up to 55% longer than normal mice.[37]

In addition to the radical extension of life span in these mice, there is other significant evidence that Ames and Snell dwarf mice age more slowly than normal mice. These include findings that there is no age-related decline in cognitive function in Ames dwarf mice;[32] that the activity of two protective antioxidant enzymes (superoxide dismutase and catalase) in the liver, kidney and brain is higher-than-normal in Ames dwarf mice;[38, 39] that Ames dwarf mice develop cancer later in life than normal;[40] that plasma glucose and insulin levels are reduced and insulin sensitivity increased in both Ames and Snell dwarf mice;[32] that Snell dwarf mice exhibit diminished osteoarthritis of the knee joint;[41] and that long-lived dwarf mice retain youthful immune function and connective tissue elasticity as they grow older.[34]

Dwarf Mice As A Model Of Extended Life Span

BioMarker is using dwarf mice as a second model of extended life span in its search for authentic anti-aging therapies. The company has been collaborating with Drs. Andrezej Bartke and Richard A. Miller to conduct microarray studies of gene expression in the tissues of dwarf mice in order to learn more about the genes involved in longevity.

The gene mutation in Ames and Snell dwarf mice is the only known way of producing anti-aging and life span-extending effects comparable to caloric restriction. The dramatic life span extension effect (up to 70%) found in these animals makes it clear that, as in CR, we are dealing with a method of retarding the aging process. The fact that only a single genetic change can extend life span so radically in mammals is strong evidence that there may only be a few key genes involved in longevity.

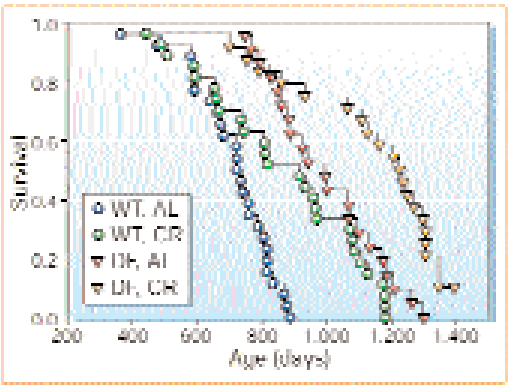

Fig. 5. Survival plots of Ames dwarf (DF) and normal (wild-type, WT) mice fed ad libitum (AL) or restricted to 70% of normal calorie intake (calorie restriction, CR). (Reproduced from Bartke A, Wright JC, Mattison JA, et al. Extending the life span of long-lived mice, *Nature*, 414:412, 22 Nov. 2001)

There is also evidence that the life span extension found in dwarf mice is somewhat different than in CR. Recently, Bartke, Miller and others showed that Ames dwarf mice, which live 50% longer than normal Ames mice, had their life span extended another 25% by CR.[42] The scientists divided 45, two-month-old Ames dwarf mice and 53 of their normal siblings into two groups, which were subjected either to CR (70% of normal food intake) or unrestricted feeding. These two groups were then followed until the animals were more than two years of age.

The CR mice had a maximum life span about 300 days longer than the unrestricted mice; the dwarf mice lived a bit longer than the CR mice; while the dwarf mice on CR lived about 500 days longer than the unrestricted mice. The survival curves in Fig. 5 show that, although both CR and dwarfism extended life span, CR reduced the rate of age-related mortality, while dwarfism shifted the age at which the age-dependent increase in mortality first became appreciable. Thus, CR appears to decelerate aging, while dwarfism appears to delay it. The scientists concluded that: "Our results indicate that long-lived Ames dwarf mice are not merely mimics of CR mice, and show that the pathways responsible for extending life span in the dwarf and CR animals are not identical."

BioMarker's preliminary gene chip results show that gene expression in the tissues of long-lived dwarf mice is similar in some respects as in the tissues of CR mice, but different in others. The company is investigating gene expression in the tissues of dwarf mice on CR. The availability of a second model of radical life extension in mammals will give BioMarker new tools in its quest to discover authentic anti-aging therapies that can also fight age-related diseases. If, as it appears, CR and dwarfism, are different in their mechanisms of action, the company will, eventually, develop anti-aging therapies that are more potent than CR mimetics alone.

BioMarker's Plans

Among the projects BioMarker is carrying out are the following:

1. Further research to explore the anti-aging, life span-extending effects of metformin, including life span studies, studies to determine the optimal dosage and administration of the drug, the search for analogs of metformin that are more potent with fewer side effects, and the combining of metformin with other drugs.

2. Research to discover other promising anti-aging, anti-disease therapies, with the same or other mechanisms of action than metformin.

3. Gene chip anti-aging research in mice, monkeys and humans. BioMarker has already started such research in monkeys and is formulating plans to do so in humans.

4. Research to pinpoint the critical anti-aging genes involved in prolonging health and extending life span.

5. Further research to determine the differences in gene expression in normal aging, delayed aging caused by CR and delayed aging caused by dwarfism, with the use of these findings to search for new and better anti-aging therapies.

6. Establishing research contracts with pharmaceutical and nutraceutical companies to test their compounds for effects on aging and age-related diseases.

7. Forming strategic alliances with major pharmaceutical companies to develop new anti-aging drugs and to discover new applications for existing drugs.

BioMarker Pharmaceuticals is the first company with a patented scientific method to measure the effects of drugs on aging in mammals (including humans). Its revolutionary technology is enabling its scientists to develop new breakthrough anti-aging therapies rapidly and inexpensively. Thus far, it has been funded exclusively by Life Extension.

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References

1. Martin GM, LaMarco K, Strauss E and Kelner KL. Research on Aging: The End of the Beginning. *Science*, 299:1339-1341, 28 Feb. 2003.
2. Ross MH. Length of life and nutrition in the rat. *J Nutr*, 75:197-210, 1961.
3. Masoro EJ. Subfield History: Caloric restriction, slowing aging, and extending life, sageke.sciencemag.org/cgi/content/fullsageke;2003/8/re2.
4. Calorie Restriction in Monkeys. *Life Extension* Magazine, July; 1998, p. 36-43.
5. Roth GS, Ingram DK, Lane MA. Calorie restriction in primates: will it work and how will we know? *J Am Geriatr Soc*, 47:896-903, 1999.
6. Walford RL, Harris, SB, Gunion, MW. The calorically restricted low-fat nutrient-rich diet in Biosphere-2 significantly lowers blood glucose, total leukocyte count, cholesterol and blood pressure in humans. *Proc Natl Acad Sci* USA, 89:11533-11537, 1992.
7. Kagawa Y. Impact of westernization on the nutrition of Japanese: changes in physique, cancer, longevity and centenarians. *Prev Med*, 7: 205-217, 1978.
8. Roth GS, Lane MA, Ingram DK, et al. Biomarkers of caloric restriction may predict longevity in humans. *Science*, 297:811, 2002.
9. Richardson A, Butler JA, Rutherford MS, et al. Effect of age and dietary restriction on the expression of alpha globulin. *J Biol Chem*, 262:12821-12825, 1987.
10. Lee CK, Klopp RG, Weindruch R and Prolla TA. Gene expression profile of aging and its retardation by caloric restriction. *Science*, 285:1390-1393, 1999.
11. Weindruch R and Walford RL. Dietary restriction in mice beginning at 1 year of age: effect on life-span and spontaneous cancer incidence. *Science*, 215:1415-1418, 1982.
12. Cao SX, Dhahbi JM, Mote PL, Spindler SR. Genomic profiling of short-and long-term caloric restriction in the liver of aging mice. *Proc Natl Acad Sci* USA, 98:10630-10635, 2001.
13. Stokkan KA, Reiter RJ, Vaughan MK, et al. Endocrine and metabolic effects of life-long food restriction in rats. *Acta Endocrinol* (Copenhagen), 125:93-100, 1991.
14. Wickner S, Maurizi MR, Gottesman S. Posttranslational quality control: folding, refolding, and degrading proteins. *Science*, 286:1888-1893, 1999.
15. Suh Y, Lee KA, Kim WH, et al. Aging alters the apoptotic response to genotoxic stress. *Nat Med*, 8:3-4, 2002.
16. McMillan DR, Xiao X, Shao L, et al. Targeted disruption of heat shock transcription factor 1 abolishes thermotolerance and protection against heat-inducible apoptosis. *J Biol Chem*, 273:7523-7528, 1998.
17. Grasl-Kraupp B, Bursch W, Tuttkay-Nedecky B, et al. Food restriction eliminates preneoplastic cells through apoptosis and antagonizes carcinogenesis in rat liver. *Proc Natl Acad Sci* USA, 91:9995-9999, 1994.
18. Yu Z, Luo H, Fu W, Mattson MP. The endoplasmic reticulum stress-responsive protein GRP78 protects neurons against excitotoxicity and apoptosis: suppression of oxidative stress and stabilization of calcium homeostasis. *Exp Neurol*, 155:302-314, 1999.
19. Warner HR. Aging and regulation of apoptosis. *Curr Top Cell Regul*, 35:107-121, 1997.
20. Nishimori H, Sasaki Y, Yoshida K, et al. The Id2gene is a novel target of transcriptional activation by EWS-ETS fusion proteins in Ewing family tumors. *Oncogene*, 21:8302-8309, 2002.
21. Lee CK, Weindruch R, Prolla TA. Gene-expression profile of the ageing brain in mice. *Nat Genet*, 25:294-297, 2000.
22. Jiang CH, Tsien JZ, Schultz PG, Hu Y. The effects of aging on gene expression in the hypothalamus and cortex of mice. *Proc Natl Acad Sci* USA, 98:1930-1934, 2001.
23. Durnas C, Loi CM, Cusack BJ. Hepatic drug metabolism and aging. *Clin Pharmacokinet*, 19:359-389, 1990.
24. Thompson PD, Hsich JC, Whitfield OK, et al. Vitamin D receptor displays DNA binding and transactivation as a heterodimer with the retinoid X receptor, but not with the thyroid hormone receptor. *J Cell Biochem*, 75:462-480, 1999.
25. Mote PL, Grizzle JM, Walford RL, Spindler SR. Age-related down regulation of hepatic cytochrome P1-450, P3-450, catalase and CuZn-superoxide dismutase RNA. *Mech Ageing Dev*, 53:101-110, 1990.
26. Rossetti L, Giaccari A, DeFronzo RA. Glucose toxicity. *Diabetes Care*, 13:610-630, 1990.
27. Lane MA, Ball SS, Ingram DK, et al. Diet restriction in rhesus monkeys lowers fasting and glucose-stimulated glucoregulatory end points. *Am J Physiol*, 28:E941-E948, 1995.
28. Harris, SB, Gunion MW, Rosenthal MJ, Walford RL. Serum glucose, glucose tolerance, corticosterone and free fatty acids during aging in energy restricted mice. *Mech Ageing Dev*, 73:209-221, 1994.
29. Spindler SR. Caloric restriction enhances the expression of key metabolic enzymes associated with protein renewal during aging. *Ann NY Acad Sci*, 928:296-304, 2001.
30. Dhahbi JM, Mote PL, Wingo J, et al. Caloric restriction alters the feeding response of key metabolic enzyme genes. *Mech Ageing Dev*, 122:35-50, 2001.
31. Dilman VM, Anisimov VN. Effect of treatment with phenformin, diphenylhydantoin or L-dopa on life span and tumour incidence in C3H/Sn mice. *Gerentology*, 26(5): 241-6, 1980
32. Bartke A, Coschigano K, Kopchick J, et al. Genes that prolong life: relationships of growth hormone and growth to aging and life span. *J Gerontol Biol Sci*, 56: 8: B340-B349, 2001.
33. Brown-Borg HM, Borg KE, Meliska CJ, Bartke A. Dwarf mice and the ageing process. *Nature*, 384: 33, 1996.
34. Flurkey K, Papaconstantiou J, Miller RA, Harrison DE. Life span extension and delayed immune and collagen aging in mutant mice with defects in growth hormone production. *Proc Natl Acad Sci* USA, 98:12:6736-6741, Jun. 5, 2001
35. Miller RA. Kleemeier Award Lecture: Are there genes for aging? *J Gerontol Biol Sci*, 54A:B297-B307, 1999.
36. Bartke A. Genetic models in the study of anterior pituitary hormones In: Shire JGM, ed, *Genetic Variation in Hormone Systems*, CRC Press, 113-126, 1979.
37. Coschigano KT, Clemmons D, Bellush LL, Kopchick JJ. Assessment of growth parameters and life span of GHR/BP gene-disrupted mice. *Endocrinology*, 14:2608-2613, 2000.
38. Hauck S, Bartke A. Effects of growth hormone on hypothamic catalase and Cu/Zn superoxide dismutase. *Free Rad Biol Med*, 28:970-978, 2000.
39. Brown-Borg HM, Rakoczy SG. Catalase expression in delayed and premature aging mouse models. *Exp Gerontol*, 35:199-212, 2000.
40. Mattison JA, Wright JC, Bronson RT, Roth GS, Ingram DK, Bartke A. Studies of aging in Ames dward mice: effects of caloric restriction. *J Am Aging Assoc*, 23:9-16, 2000.
41. Silberberg R. Articular aging and osteoarthritis in dwarf mice. *Path Microbiol*, 38:417-430, 1972.
42. Bartke A, Wright JC, Mattison JA, et al. Extending the life span of long-lived mice. *Nature*, 414:412, 22 Nov. 2001.
43. Corpas E, Harman SM, Blackman MR. Human growth hormone and human aging. *Endocr Rev*, 14:20-39, 1993.
44. Rudman D, Feller AG, Nagraj HS, et al. Effects of human growth hormone in men over 60 years old. *N Engl J Med*, 323:1-6, 1990.
45. Aleman A, Verhaar HJJ, De Haan EHF, et al. Insulin-like growth factor-1 and cognitive function in healthy older men. *J Clin Endocrinol Metab*, 84:471-475, 1999.
46. Baum HBA, Katznelson L, Sherman JC, et al. Effects of a physiological growth hormone (GH) therapy on cognition and quality of life in patients with adult-onset GH deficiency. *J Clin Endocrinol Metab*, 83:3184-3189, 1998.
47. Longo VD, Finch CE. Evolutionary medicine: from dwarf model systems to healthy centenarians? *Science*, 299:1342-1346, 28 February 2003.