Workshop Report

Biomarkers of Aging: From Primitive Organisms to Man

An Interdisciplinary Workshop of the
INTERNATIONAL LONGEVITY CENTER–USA

Sponsored by
The Ellison Medical Research Foundation
Kronos Longevity Research Institute
Institute for the Study of Aging
Canyon Ranch Health Resort

ILC

INTERNATIONAL
LONGEVITY CENTER–USA

Biomarkers of Aging: From Primitive Organisms to Man

An Interdisciplinary Workshop of the
INTERNATIONAL LONGEVITY CENTER–USA

Sponsored by
The Ellison Medical Research Foundation
Kronos Longevity Research Institute
Institute for the Study of Aging
Canyon Ranch Health Resort

Tucson, Arizona – October 5-8, 2000

Participants

CO-CHAIRS

Robert N.Butler, M.D.
International Longevity Center–USA

Richard L. Sprott, Ph.D.
The Ellison Medical Foundation

PARTICIPANTS

Jeffrey Bland, Ph.D.
Metagenics

Richard Feuers, Ph.D.
National Center for Toxicology Research

Michael Forster, M.D.
Texas College of Osteopathic Medicine

Howard Fillit, M.D.
Institute for the Study of Aging, Inc.

S.Mitchell Harman,M.D., Ph.D.
Kronos Longevity Research Institute

Michael Hewitt, Ph.D.
Canyon Ranch Health Resort

Mark Hyman, M.D.
Canyon Ranch Health Resort

Kathleen Johnson,M.S., R.D.
Canyon Ranch Health Resort

Evan Kligman, M.D.
University of Arizona

Gerald McClearn, Ph.D.
Pennsylvania State University

Richard Miller, M.D., Ph.D.
University of Michigan

James Nelson, Ph.D.
University of Texas-San Antonio

Arlan Richardson, Ph.D.
University of Texas-San Antonio

William Sonntag, Ph.D.
Wake Forest University

Huber Warner, Ph.D.
National Institute on Aging

Richard Weindruch , Ph.D.
University of Wisconsin

Norman Wolf, D.V.M., Ph.D.
University of Washington

Preface

By Robert N. Butler, M.D. and Richard L. Sprott, Ph.D.

The International Longevity Center and The Ellison Medical Foundation, joined by the Kronos Longevity Research Institute and the Institute for the Study of Aging, developed a workshop at Canyon Ranch Health Resort, where we assembled some of America's leading biologists and clinicians interested in aging to consider what we presently know about putative biomarkers of aging from rodents to man. Our goals were to come to a consensus, construct an agenda for future research, and make appropriate recommendations to policy-makers and the public-at-large.

These efforts are extremely important because of well-publicized claims that the means exist to directly intervene in aging processes. Obviously, to verify such claims and test interventions experimentally it will be necessary to have proven indicators which can monitor the aging processes, just as pressure readings are used to monitor cardiovascular health.

These considerations follow a significant scientific heritage which has resulted in an attempt to separate the normative, multi-causal processes of aging from diseases and other factors. It is understood that chronological age remains the most valid, if imperfect, biomarker of aging, and that aging per se is a risk factor for a variety of diseases, largely the polygenic conditions of late life such as Alzheimer's disease, cancer and coronary heart disease.

However, it is self-evident that chronological age cannot be used as a biomarker in the sense described above, because any intervention that is successful in slowing the aging process will, by design, result in asynchrony between biological and chronological age.

These thoughts proved critical to the results of the workshop. The biologists attending were able to describe in extraordinary detail the failure as yet to find validated biomarkers of aging in either rodents or man. At the same time, the clinicians were able to identify disease markers, risk factors and functional measures that show dramatic correlations with longevity and quality of life.

Aging processes are manifest in the famous Gompertz Curve, described in 1825 by a London actuary, Benjamin Gompertz. It shows the rise in the "force of mortality" with the passage of time. This important observation demonstrates profoundly why it is necessary to provide major funding for studies at the molecular and cellular level so that we may better understand what within our bodies increasingly predisposes us to disease, disability and ultimately death as we grow older.

At the same time, at an accelerated pace in the last half century, we have learned about many factors that adversely affect health, longevity and quality of life.The ability to recognize disease markers, risk factors and measurable functional activities offers enormous power to the clinician.

Thus, the workshop report that follows is divided into two parts. First, the biology of aging and the studies of biomarkers are described. Reasons

are given why there is no validity to current claims that a person's "real age" can be measured, and it is emphasized that such services should not be marketed to the unwitting public.

The second part describes some varied disease markers, risk factors and functional measures which do offer useful information to clinicians, and can help people alter their lifestyles to maintain or improve their health. These measures are critical "wake-up calls" to promote health and avoid unnecessary illness. ■

Workshop Overview

INTRODUCTION

What biological changes take place as we age? Efforts by scientists to uncover the biomarkers of aging, that is, the normal phenomena of growing old, and to separate these inevitable physiologic changes from diseases and other factors have yielded tantalizing clues but few definitive answers. We know that there are age-related risk factors for a number of diseases of older life, such as Alzheimer's disease. However, we do not know if they are simply *by-products* of aging, or if they are an *essential component* of the aging processes. Nor do we know how long and how well physiological functions can be maintained with increasing age. Finally, to date we cannot separate genetics, lifestyles, the environment and other idiosyncratic factors from age-related functions that are universal.

The biological markers of diseases and factors that make individuals vulnerable to certain diseases will enable clinicians to develop interventions to increase life expectancy and/or enhance function in aging populations. Biological markers of aging are also extremely important because of well-publicized claims that the means exist to retard or reverse aging processes. Obviously, to verify such claims and test putative interventions experimentally it will be necessary to have proven indicators which can monitor aging processes.

The International Longevity Center and The Ellison Medical Foundation, joined by the Kronos Longevity Research Institute and the Institute for the Study of Aging, held a workshop at Canyon Ranch Health Resort and assembled some of America's leading biologists and clinicians interested in aging to consider the question of whether biomarkers can be identified and used to measure the physiological age of any individual within a population,given emerging information about aging and new technological advances. Our goals were to come to a consensus, construct an agenda for future research, and make appropriate recommendations to policy-makers and the public-at-large.

A Definition of Aging

Our workshop report begins with a working definition of aging. Caleb Finch[1] offers a good overall definition: "A nondescript colloquialism that can mean any change over time, whether during development, young adult life, or senescence. Aging changes may be good (acquisition of wisdom); of no consequence to vitality or mortality risk (male pattern baldness); or adverse (arteriosclerosis)".

This report, however, focuses only upon the adverse aspect of aging: the processes that progressively convert physiologically and cognitively fit healthy adults into less fit individuals with increasing vulnerability to injury, illness and death. We are particularly interested in the changes organisms undergo that adversely affect their vitality and functional health over most of the adult life span.

[1] Longevity, Senescence, and the Genome.Caleb E. Finch. Chicago:University of Chicago Press,1990,671.

The Force of Mortality

While aging per se is a risk factor for a variety of diseases, such as Alzheimer's disease, cancer and coronary heart disease, chronological age cannot be used as a biomarker in the sense described above, because any intervention that is successful in slowing the aging processes will, by design, result in asynchrony between biological and chronological age.The famous Gompertz Curve, described in 1825 by a London actuary, Benjamin Gompertz, shows the rise in the "force of mortality" with the passage of time, which is the manifestation of the underlying biology of aging.

Clinical Criteria for Biomarkers

In the absence of a more complete understanding of the mechanisms of aging, clinicians would like to have age-related biomarkers that have adequate predictive value to provide information to their patients. This information could help improve organ function throughout the life cycle, and reduce unnecessary morbidity and premature mortality. These biomarkers must be more than disease risk factors, and represent individual indicators of functional status. Clinicians prefer functional biomarkers that relate to health expectancy, and that

1. Predict physiological, cognitive and physical function in an age-coherent way, and do so better than chronological age;

2. Predict the years of remaining good function, and the trajectory toward organ-specific illness in the individual;

3. Are minimally invasive, and accessible to many individuals.

Several types of data could be utilized, including anthropometrical data, such as body mass index, body composition, and bone density; functional challenge tests, such as glucose tolerance test and forced vital capacity; and physiological tests, such as cholesterol/HDL.

These biomarkers could be measured in a large group of people who have reached an age where functional loss is known to occur most rapidly (i.e., the 60 to 70 age group), but it would also be useful to have data on younger adults as well. Analyses would help to identify tests whose predictions were most accurate when matched against actual functional outcome and morbidity patterns. Tests with the best predictive value would become functional biomarkers. They could be used to test specific clinical approaches and therapies that focus on improvement of physiological, cognitive and physical functioning and their relationship to functional age.The optimal goal would be to obtain functional biomarkers with which personalized medicine or other interventions could be developed, to effectively reduce morbidity and improve organ-specific function. Achieving this goal would delay the necessity for costly hospitalization or social support of the aging population.

Ongoing Research

A number of studies have advanced our knowledge, at the same time as they raise provocative questions:

- It is well-documented that growth hormone levels fall with increasing age. Does this mean that low growth hormone levels accelerate aging? Not necessarily. It is equally plausible that falling growth hormone levels may merely reflect other aging processes which lead to dysregulation of a variety of cells, including cells that secrete growth hormone and those that regulate their secretion. In fact, lower growth hormone levels may be an indicator of health. Research findings on mice that overproduce growth hormones indicate that they live only a short time, suggesting that growth hormone deficiency per se does not cause accelerated aging, but that the opposite may be true[2].

[2] Bartke A,Brown-Borg H,Mattison J, et al.2001. Prolonged longevity of hypopituitary dwarf mice.Exp. Gerontol.36:21-28.

- In 1935, Clive McCay first reported the effects of aging and caloric restriction in rats and mice. Today, there is a body of literature which shows how caloric restriction alters age-related pathology [3,4.]

- Research suggests that the nervous system is a critical factor in regulating the life span in laboratory worms (nematodes), and that mutations in a specific gene can result in dramatic life span extension[5]. We do not know if the nervous system of mammals is similarly implicated, and if so, how this occurs.

- Studies have shown that chromosomes become shorter each time a human cell divides, as their ends are removed and not replaced[6]. These end regions, known as telomeres, should at least be considered as a possible biomarker of human aging. While telomere length is an indicator of how many times a human cell has divided, rather than a direct indicator of aging per se, it can be an indicator of functional age in certain human cells or in tissues where replicative potential is crucial to function, such as fibroblast involvement in wound healing.

- Imaging techniques, including nuclear magnetic resonance (NMR) and positron emission tomography (PET), hold particular promise in overcoming some of the technical problems associated with studies of aging over extended periods of time (longitudinal studies). With the recent development of high-resolution cameras capable of imaging small animals, it is now possible to perform relatively non-invasive studies on rats and mice as they age. Functional NMR can be used to study the changes in anatomy and metabolic activity in the brain and other tissues during aging. PET imaging may be used to study the neurochemical changes that occur in the brain during aging, including changes in neurotransmitter receptors and neurotransmitter synthesis.

[3] Lipman RD, Dallal GE,and Bronson RT. 1999a. Lesion biomarkers of aging in B6C3F1 hybrid mice. J. Gerontol.54A:B466-477.

[4] Lipman RD, Dallal GE,and Bronson RT. 1999b. Effects of genotype and diet on age-related lesions in *ad libitum* fed and calorie-restricted F344,BN and BNF3F1 rats. J. Gerontol.54A:B478-491.

Hurdles to Establishing Biomarkers

There are several hurdles to establishing informative biomarkers. One is the biological variation between individuals, which makes generalizations difficult. Another is the overlapping of aging and disease processes. Other hurdles include our uncertainty about which age-related changes are benign and which are indicators of pathology; we do not have enough information to determine if there is a point at which a process begins to do damage to the organism, and if so, the point at which it occurs; we do not know when to distinguish critical from non-critical damage. Finally, and significantly, it is difficult to obtain funding for this research.

Policy Implications

Obtaining support for a biomarker research agenda presents serious problems. The research program which was supported for 10 years (1988 – 1998) by the NIA was accomplished through set-aside funds and use of an ad hoc review process. Review of applications for biomarker research by regular Center for Scientific Review peer review groups at the NIH is not likely to result in sufficient numbers of funded applications to make substantial progress in this area in the near future because their focus is on the underlying mechanisms of diseases. Research on bio-markers does not address this concern. Clearly, a non-traditional long term source of funding is required, possibly involving commercial or philanthropic sources of support. However, as long as the Food and Drug Administration has no program for evaluating putative anti-aging interventions, commercial organizations are unlikely to perceive sufficient pay-off for funding such aging research.

[5] Larsen PL,Albert PS,and Riddle DL.1995.Genes that regulate both development and longevity in *Caenorhabditis elegans.* Genetics 139:1567-1583.

[6] Harley CB, Futcher AB,and Greider CW. 1990. Telomeres shorten during ageing of human fibroblasts. Nature 345:458-460.

[7] Shelton, D. "Dipping into the fountain of youth," *American Medical News,* December 4,2000.

Public Education

Leadership of the American Academy of Anti-Aging Medicine and others in the USA would have us believe that aging is not inevitable, and that "immortality is within our grasp"[7]. They believe that well-validated biomarkers of aging already exist which can be used to evaluate individuals at a cost of several thousand dollars per person, and that these evaluations can then be used to design individualized anti-aging treatments. Unfortunately these treatments include some poorly validated interventions such as improving anti-oxidant status and hormone replacement therapies, (e.g., growth hormone, testosterone, dehydroepiandrosterone [DHEA], melatonin, etc.)

While it is seductive to believe that restoring hormone levels back to levels that are produced in young persons is a good thing, and although it is true that hormone replacement trials have yielded some positive short term results, it is clear that negative side effects also may occur, in the form of increased risk for cancer, cardiovascular disease, behavior changes, etc. Estrogen replacement therapy in women has shown definite benefits, especially for prevention of osteoporotic fractures. Nonetheless, research on this hormone is still underway, and some recent studies have raised "red flags" with regard to the usefulness of estrogen for treating or preventing coronary heart disease. The risk/benefit ratios for testosterone replacement and growth hormone treatment have not been established in older persons. Finally, trials of DHEA have failed to show significant clinical benefits in normal aging. Clinical trials to investigate the risks and benefits of these and other potential interventions are either still going on, or have not yet provided definitive answers. The public is advised to exercise caution in requesting these popular anti-aging interventions until adequate clinical trials have been completed and analyzed.

[7] Shelton, D. "Dipping into the fountain of youth," *American Medical News*, December 4,2000.

The participants of this workshop strongly recommended continuing research on these and other hormones, antioxidants and agents that may have favorable effects upon the promotion of health, e.g., the possibility that some anabolic hormones protect, if only for a short term, against the frailties of old age. At the same time, advancement of healthier lifestyles with attention to diet, exercise, tobacco cessation and early identification of risk factors, measurements of functional status and disease markers are desirable and achievable goals. For example, it is important to lower cholesterol levels through exercise or the use of pharmacological agents like statins, and to detect hypertension and diabetes early in order to effect appropriate control and prevent the often lethal consequences of both.

Conclusion

Early identification of risk factors, measurements of functional status and disease markers are desirable and achievable goals, and new studies are advancing our understanding of factors that contribute to health and longevity, among them exercise, smoking cessation, nutrition, environmental and genetic factors. At the same time, our rapidly aging population increases the relevance of research to find age-related biomarkers. Although a definitive panel of biomarkers for assessing physiological age of individuals within a population has not yet been achieved, studies using organisms in the laboratory continue to provide researchers with important data. The ultimate goal is to develop age-related biomarkers to measure interventions that may increase life expectancy and enhance healthy aging for as long as possible. ■

The Workshop Report

INTRODUCTION

A discussion about biomarkers of aging immediately runs into some difficulty, first because few people can agree on a definition of aging, and second, because different definitions of "biomarker" are employed by basic and clinical scientists with different interests and backgrounds. Edward Masoro pointed this out in 1988 when he wrote that "there are two major reasons why there is controversy about the use of physiological systems as biomarkers of aging: one relates to the lack of knowledge about the basic aging processes and the other is the confusion about what a biomarker of aging is designed to do" (Masoro, 1988). Leaving aside for the moment the question as to whether such barriers to biomarker development are insurmountable, we must begin with a working definition of aging. One good overall definition is that aging is "A nondescript colloquialism that can mean any change over time, whether during development, young adult life, or senescence. Aging changes may be good (acquisition of wisdom); of no consequence to vitality or mortality risk (male pattern baldness); or adverse (arteriosclerosis)" (Finch, 1990). For the purposes of this discussion however, we will focus upon the adverse aspect of aging: the process that progressively converts physiologically and cognitively fit healthy adults into less fit individuals with increasing vulnerability to injury, illness and death. We are particularly interested in the changes in an organism that adversely affect its vitality and functions over most of the adult life span.

At the workshop, biomarkers of aging were defined by participant Richard Miller as traits which meet three criteria:

1. The biomarker should predict the outcome of a wide range of age-sensitive tests in multiple physiological and behavioral domains, in an age-coherent way, and do so better than chronological age;
2. It should predict remaining longevity at an age at which 90% of the population is still alive, and do so for most of the specific illnesses that afflict the species under study;
3. Its measurement should not alter life expectancy or the outcome of subsequent tests of other age-sensitive tests.

This definition provided a framework for the discussion at the workshop.

The second criterion implies that biomarkers are likely to be measuring degenerative processes, not just age-related change. Some effects of age, such as experience and judgment, may be beneficial, but are unlikely to pass the second criterion. Others, such as gray hair or skin wrinkles, may themselves have little effect on mortality risks, yet still serve as easily measurable indices of underlying degenerative processes that do increase vulnerability.

A continuing controversy is whether there exist processes of aging per se, which can be identified and studied independently of age-related disease. It is clear that there are age-related risk factors for

disease, and that these overlap with risk factors for aging, but there is disagreement about whether diseases to which older persons are vulnerable should be considered merely by-products of aging, or an essential component of the aging processes. This seems to be primarily a semantic issue for some, but a major question for others, and the issue cannot be settled here. What is important is how long and how well physiological functions can be maintained with increasing age; whether and what measurements can be done to assess this biologically, and in so doing obtain a multi-component physiological yardstick for aging. Ultimately, the goal is to use this tool to develop interventions that increase life expectancy and/or enhance function in aging populations.

NIA-SPONSORED WORKSHOPS IN 1981 AND 1986, AND THE 1988-1998 BIOMARKERS INITIATIVE

This is at least the third workshop on Biomarkers of Aging. In 1981 the National Institute on Aging organized its first conference on nonlethal biological markers of physiological aging. A second workshop, also sponsored by the National Institute on Aging (NIA), was held in 1986 in Chicago, Ill. It was convened to discuss "strategies for the conduct of biomarkers of aging research prior to the initiation of a request for biomarker research applications by the National Institute on Aging. The intent of the NIA was to generate interest in biomarker research, update general understanding of the biomarker concept, and most important, solicit the advice of knowledgeable scientists before issuing requests for research applications in this area" (Sprott, 1988). Such a request for applications was issued by the NIA in 1987, and applications were funded beginning in Fiscal Year 1988.The program was renewed for five more years in 1993, and continued until 1998. Although the research was done on genetically homogeneous strains of rats and mice, the hope was that any panel that was developed might also be relevant to human populations which are genetically heterogeneous.

This 10-year initiative resulted in many publications, but it appears that a definitive panel of biomarkers for assessing the physiological age of individuals within a population was not achieved. A series of seven papers was published in the November and December, 1999 issues of the Journal of Gerontology (v.54A, p.B464-566). These reports are among the first to summarize the results of this broad initiative (Sprott, 1999). They include a comprehensive summary of the age-related pathology observed in the rats and mice used in this study and how caloric restriction alters it (Lipman et al., 1999a, 1999b), as well as an extensive characterization of growth and survival characteristics of the various mouse and rat models used (Turturro et al., 1999). The remaining four papers describe a variety of attempts to identify and/or validate various biomarkers of aging, such as age-related changes in the potential for cell proliferation (Wolf and Pendergrass, 1999), changes in circulating hormones (Sonntag et al., 1999) and brain MAPK signaling (Zhen et al., 1999), and behavioral changes (Markowska and Breckler, 1999). The work supported by this NIA biomarker initiative thus added to the literature documenting the effects of aging and caloric restriction on a variety of interesting traits, but did not produce convincing evidence that these candidate biomarkers, separately or in combination, provided information about the "physiological age" of the individual upon whom the measurements were done.

2000 WORKSHOP

The purpose of this most recent workshop was to re-visit the question of whether biomarkers of aging can be identified and used to measure physiological age of any individual within a

population, given emerging information about aging and new technological advances. The meeting was organized by Robert Butler and Richard Sprott, and the participants included several individuals involved in the 1988-1998 initiative (Richard Feuers, Michael Forster, William Sonntag, Norman Wolf), several gerontologists not involved in the initiative (Jeffrey Bland, Michael Hewitt, Gerald McClearn, Richard Miller, James Nelson, Arlan Richardson and Richard Weindruch), and several clinicians (Howard Fillit, Mitchell Harman, Mark Hyman, Kathleen Johnson and Evan Kligman).

Their discussions centered on the following issues:

- What are the hurdles to evaluating and validating biomarkers of aging?
- Is the central nervous system a pacemaker of aging?
- Development of a research agenda
- Identification of possible interventions that might alter aging and delay age-dependent pathology
- Overlap between "biomarkers of aging" and "indicators of functional status"
- Policy implications
- Public education

What are the hurdles to evaluating and validating biomarkers of aging?

There are several hurdles to establishing informative biomarkers. One is the inter-individual and measurement variations which could be large enough to obscure differences due to aging-related change. Another is the overlapping of aging and disease processes as sources of change. Other hurdles include our uncertainty about which age-related changes are benign and which are indicators of adverse events; our lack of information about whether there are damage thresholds which only have a significant effect once these thresholds are breached, and if so what these thresholds are; our need to distinguish critical damage from non-critical damage (e.g., mutations need not lead to amino acid changes in proteins, and not all oxidized side chains in proteins will have functional consequences). Finally, there is the practical hurdle of obtaining support for the research needed; grant applications, including proposals to identify and validate biomarkers are unlikely to be enthusiastically reviewed by the usual peer review process, because of the perceived non-mechanistic nature of such research.

Is the central nervous system a pacemaker of aging?

Several recent publications describing research on *Caenorhabditis elegans (C. elegans)* suggest that the nervous system is a critical factor in regulation of life span in nematodes. Mutations in the *daf-2* gene in nematodes can result in dramatic life span extension (Larsen et al., 1995). The *daf-2* gene codes for an insulin receptor-like protein (Kimura et al., 1997; Wolkow et al., 2000) recently showed that restoring *daf-2* function in the neurons alone was sufficient to specify wild type life span, whereas a similar intervention in muscle or intestine had no such effect. The nervous system in nematodes has also been implicated in life span regulation by Apfeld and Kenyon (1999), who showed that mutations blocking sensory signal transduction extend nematode life span. Ailion et al. (1999) showed that mutations in *unc-64* extend nematode life span, and that the site of action of *unc-64* is neuronal, and through the insulin receptor pathway. Finally, overexpression of human Cu/Zn superoxide dismutase (SOD-1)

in motor neurons in fruit flies also extends life span (Parkes et al., 1998). Thus, this series of findings clearly implicate the nervous system in life span regulation in these two invertebrate systems, but the question remains whether, and how, the mammalian nervous system might be similarly implicated.

In the search for meaningful biomarkers of aging, the mammalian neuroendocrine system presents a more confusing picture. One interesting place to look might be regulation of either growth hormone (GH) production or function, because it is well-documented that circulating GH levels fall with increasing age, which suggests that low GH levels might accelerate aging. However, it is equally likely that falling GH levels may merely reflect one or more underlying aging processes which lead to dysregulation of differentiated cells of various types, including those that secrete, and those that regulate the secretion of GH. Moreover, there are several lines of evidence that suggest that GH deficiency per se is not a cause of accelerated aging, and that the opposite may be true.These include the following: mice overproducing GH are short-lived (Bartke et al., 2001); mice selected for slow growth rates in the first two months of life are relatively long-lived (Miller et al., 2000); dwarf mutant mice (*df* and *dw* mutations) with defects in GH, prolactin, and thyroid stimulating hormone production, have extended longevity (Brown-Borg et al., 1996; Miller, 1999), as do GH receptor-deficient mice (Coschigano et al., 2000); and the inverse correlations between body size and life span in mice and dogs (Miller, 1999).These *df* and *dw* mice have defects in pituitary development, and as a result exhibit multiple endocrine deficiencies. It is not known which deficiency, if any, is critical for life span extension, but it is worth noting that GH receptor-deficient mice are neither thyroid nor prolactin-deficient.

One possible new tool for looking at age-related changes in brain function is gene expression microarray technology. Lee et al. (2000) have reported a first experiment to investigate such changes in mouse cerebellum and neocortex using arrays representing 6,347 genes. Their general conclusion was that aging-related changes in these tissues are indicative of increased oxidative stress and an inflammatory response with increasing age. However, it is too early to know how useful microarrays will be in identifying informative transcriptional biomarkers of either brain function or aging, and if they are, which genes will be critical. Finally, the use of neuroimaging technologies is also promising for the development of brain-related biomarkers. Imaging techniques can be used to estimate changes in brain activity, and thus indirectly cell number. Significant reduction of cell number in brain, or other critical tissues, might predict physiological age and mortality. These new tools will be briefly addressed in the next section.

Development of a Research Agenda

The 1988-1998 NIA Biomarkers of Aging Initiative was based on the idea that biomarkers would be modulated by caloric restriction (CR) intervention. It still seems reasonable that at least some physiological indicators of aging may be so modulated, as CR remains the only known intervention to reliably retard aging and extend maximum life span in a wide variety of species (Masoro, 2000). Of some relevance is the recent observation that the expression of only about two per cent of mouse genes in post-mitotic tissues are changed by two-fold or more during aging in mice, and that many, but not all, of these age-related changes are reversed by CR (Lee et al., 1999; Lee et al., 2000). In fact, incomplete reversal of age-related changes in gene expression by CR may provide insights into which changes are critical in aging (Han et al., 2000).

If one assumes that genes whose expression changes with age are likely to be associated with informative biomarkers of aging, then it becomes important to ask what is the potential for gene expression microarray analysis in biomarker research using mice? Such an approach might require two stages (Miller et al., 2001).The first stage would be to test all known mouse genes for changes in expression greater than some arbitrary amount, say 50% or 100% change, using enough mice to achieve statistical significance. Further levels of complexity of such an undertaking are that, 1) many genes are expressed in a tissue-specific manner, so that multiple tissues would have to be examined separately; 2) it will be necessary to follow the sequence and patterns of changes over a range of ages, rather than to simply examine animals arbitrarily defined at two age points as young and old; and, 3) it will be necessary to examine changes in several strains of mice, because some apparent aging changes may turn out to be strain-specific. Although the complete sequence of the mouse genome is not yet known, the sequence is expected to become available in the next 2-3 years. As various DNA-based microarray technologies improve, there is optimism that changes of as little as 20% may be reliably detected (personal communication, Minoru Ko, Gerontology Research Center, Baltimore, MD). Once this has been done, the expression of all qualifying genes, that is, genes showing statistically significant age-related changes of at least some minimum magnitude in more than one strain, would need to be re-examined as a function of tissue and at a variety of ages, and these changes related to development of pathology, to identify which changes in gene expression might be informative. Unfortunately the invasive nature of such an experiment precludes its use in longitudinal studies for most tissues, so the remaining life span of the individual mouse could not be determined. However, cross sectional results should identify some small number of genes whose expression changes substantially enough with increasing age to be a putative biomarker of the condition of some physiologically important system(s).

Just how many genes will be identified in this way depends upon the sensitivity and reliability of the microarray system used, and the amount of biological variation inherent in the expression of each gene (Dozmorov et al., 2001). It will also depend on the percent change and statistical significance limits imposed in the first phase. The results of Lee et al. (1999, 2000) suggest that the theoretical maximum number of mouse genes would be no larger than about 1,000 genes for any given tissue, assuming there is a total of about 50,000 mouse genes and that both increases and decreases are relevant. Major caveats to this approach include the potential high variability among results obtained from genetically heterogeneous individuals; the possibility that highly relevant "age indicators" may lie below the detection limit in such an analysis; and the invasive sampling procedure required. Nevertheless, DNA-based microarray technology is potentially very powerful, and as the reliability and sensitivity of the technology improves, it should eventually become useful in evaluating the physiological status of aging animals and/or humans. Future development of protein-based microarray technologies for screening the amount and activity of specific proteins may turn out to provide an even better approach (MacBeath and Schreiber, 2000).

The caveats discussed above apply as well to the validation of any potential biomarker of aging. However, each type of potential biomarker will also present its own unique hurdles.There is no doubt that aging and age-related pathology are accompanied by oxidative damage, but it is less clear which oxidative modifications are critical. The presence of 8-hydroxyguanine in DNA and

amino acids with oxidized side chains in proteins are generally accepted biomarkers of oxidative stress, but it is not clear whether global measurements of oxidative stress are sufficiently informative to provide biomarkers of aging. Techniques for measuring levels of 8-hydroxyguanine in DNA are much improved over those used 5-10 years ago, but it is not yet clear how good an indicator of aging they may be. Pero et al. (2000) have suggested that as crude a measurement as serum protein sulfhydryl groups correlate with mammalian life span. A more promising approach might be to identify proteins which are essential for a critical function, such as ATP production, and may become rate-limiting through oxidative or other damage. Two examples of this are cis-aconitase (Yan et al., 1997), and adenine nucleotide translocase (Yan and Sohal, 1998). Two other candidates are glutamine synthetase (Carney et al., 1991), which detoxifies ammonia while lowering glutamate levels in the brain, and poly ADP-ribose polymerase (Pero et al., 2000), which is essential for DNA repair in eukaryotic systems.

If aging is at least partially reflected in a loss of ability to maintain homeostasis, then a decrease in one or more stem cell populations might predict there is less life span remaining, especially if these stem cells are critical for replacement of cells lost through apoptosis. However, no direct evidence exists to suggest that this is so, and good methods for isolating and characterizing stem cells are not yet available. In a similar vein, some measure of DNA repair capacity might predict the ability to maintain genetic stability, and thus homeostasis. Although DNA damage is most frequently associated with cancer risk, a defective Werner's syndrome gene leads to genetic instability and some aspects of aging prematurely, as well as increased tumorigenesis (Oshima, 2000).The Werner's syndrome gene product may very well be involved in DNA repair as it codes for both DNA helicase and 3' exonuclease activities, and loss of these two activities appears to be related to premature aging.

Studies have shown that chromosomes become shorter each time a human cell divides, as their ends are removed and not replaced. (Harley et al., 1990) These end regions, known as telomeres, should at least be considered as a possible biomarker of human aging. While it is clear that telomere length is an indicator of how many times a human cell has undergone cell division rather than a direct indicator of aging per se, it might be informative as an indicator of functional age in certain human cells or tissues where replicative potential is crucial to function, e.g., fibroblast involvement in wound healing. However, because of their initially long telomere length, rodent cells appear not to rely on telomere length-induced replicative senescence to limit the number of cell divisions available (Shay and Wright, 2001).Thus, attempting to validate telomere length as a biomarker in rodent cells may not be useful in developing a human biomarker for aging. However, there are reports that telomere length does decrease and might be correlated with aging in some rat tissues (Jennings et al., 1999; Kajstura et al., 2000).

A major problem with the above suggestions is that most require some invasive sampling, and thus are likely to violate criterion number three. Non-invasive sampling and measurements are much more desirable, which would limit experimentation to blood samples, anthropometric measurements, imaging techniques, or possibly skin, muscle or fat biopsies. Another problem is that they depend on correct guesses about candidate biomarkers, which earlier experience suggests have only a limited chance of success. A real biomarker validation program could be constructed by encouraging a substantial number of laboratories (perhaps 10?) to measure

overlapping sets of 10-25 biochemical, physiological or psychological traits, depending on the expertise of the laboratory, in several hundred genetically heterogeneous mice at several ages, and coupling these measurements with data on survival and pathology assessment at death. These data should be provided in a form suitable for statistical analysis to identify significant correlations among age-sensitive traits, and predictive value for life span and a variety of age-related diseases. Pre-existing data sets like the Baltimore Longitudinal Study of Aging and the Framingham longitudinal studies should also be mined for analogous traits in humans. Also, genetic studies on centenarians may increasingly identify both favorable and unfavorable alleles for promoting long life (Perls et al., 2000; Perls, 2001). These combined approaches should identify some promising biomarkers to be validated prospectively in human studies.

Merely showing that a given assay changes with age, and thus distinguishes most old people from most young people, is not sufficient to qualify a test as a biomarker. There are, and will continue to be, many candidates for biomarkers, but the real challenge in developing a productive research agenda is to validate some of these as true biomarkers. The test in question must divide people (or mice) of a given age into groups that differ predictably in a wide range of other age-sensitive traits (Miller, 1997).

Imaging techniques, including nuclear magnetic resonance (NMR) and positron emission tomography (PET), hold particular promise in overcoming some of the technical problems associated with longitudinal studies of aging. With the recent development of high-resolution cameras capable of imaging small animals, it is now possible to perform relatively non-invasive studies on rats and mice as they age. Functional NMR can be used to study the changes in anatomy and metabolic activity in the brain and other tissues during aging. PET imaging may be used to study the neurochemical changes that occur in the brain during aging, including changes in neurotransmitter receptors and neurotransmitter synthesis. A drawback of these procedures in animal studies is the need to anesthetize the animals, and proximity to the necessary imaging facilities. An exciting new use for PET imaging is the non-invasive imaging of reporter gene expression in living animals (Herschman et al., 2000). Using PET reporter genes and PET reporter probes investigators can examine the transcriptional activity and activation of promoters incorporated in transgenes or in viral vectors. One enormous potential advantage of non-invasive imaging of gene expression in living animals is that repeated analysis of gene expression could be made during experimental manipulations. With the rapid advancements in this area, it is quite possible that imaging techniques will become available that will allow scientists to monitor non-invasively, in real time, the levels of reactive oxygen species in tissues and groups of cells. This technology is becoming extremely important in aging research, especially in studies with human subjects (Bookheimer et al., 2000; Small et al., 2000).

Identification of Possible Interventions

One of the major reasons for identifying and validating biomarkers would be to obtain endpoints for testing possible interventions in a model system to retard, prevent, or even reverse adverse age-related changes, as discussed by Warner et al. (2000).These authors concluded that a comprehensive panel of informative endpoints in mice might include survival curves; pathology assessment; non-invasive endpoints such as locomotion, cognitive function, physiological function (e.g., T-lymphocyte subsets);

biomarkers of oxidative stress; other measures of resistance to stress; and gene expression microarray analysis. However, first these endpoints need to be validated as to their value as true biomarkers in such a testing program.

Although antioxidant interventions continue to be a favorite choice for testing, the success of such interventions has been mixed despite some epidemiological data suggesting that dietary vitamin E supplementation reduces the risk of heart disease in men and women (Rimm et al., 1993; Stampfer et al., 1993). Life span extension has been observed in invertebrate systems overexpressing Cu/Zn superoxide dismutase (SOD) (Parkes et al., 1998; Sun and Tower, 1999), but this is not a viable human intervention. However, Melov et al. (2000) have recently shown that a SOD/catalase mimetic called EUK-134, when added to the diet, does extend life span in nematodes, and using this compound in humans might be possible. In contrast, Richard Weindruch reported at the workshop that in his research laboratory no life span extension occurred in male middle-aged mice treated with a variety of compounds including a-lipoic acid, N-acetyl cysteine, vitamin E, coenzyme Q10, melatonin, and aminoguanidine, alone and in various combinations. However, these negative results do not preclude the possibility that some of these interventions might retard one or more organ-specific aging processes in either mice or humans.

A very recent paper suggests that genetically-induced reduction of the transport of dicarboxylic acids, key intermediates in the citric acid cycle, appears to slow aging in fruit flies (Rogina et al., 2000). This mutation could be mimicking one aspect of caloric restriction, which could possibly also be accomplished pharmacologically by using an inhibitor of this dicarboxylic acid transport enzyme.

It is widely accepted that mitochondria are the chief source of reactive oxygen species (ROS) in eukaryotic cells. Although it is not known exactly how much superoxide anion is generated by mitochondria during normal oxidative metabolism, estimates are in the range 1% - 5% of the total oxygen consumed by the electron transport system. This superoxide is converted to hydrogen peroxide by the mitochondrial Mn-superoxide dismutase. However, hydrogen peroxide itself is a reactive compound, and may leak into the cytoplasm, where it can peroxidize fatty acids in membranes or be converted to hydroxyl radical which rapidly damages proteins and nucleic acids.The enzyme catalase is necessary to convert this hydrogen peroxide into harmless oxygen and water. Also relevant is the discovery that cytochrome C leaking from damaged mitochondria is a triggering event for apoptosis (Green and Reed, 1998).This sequence of events is particularly damaging in post-mitotic tissue, where the potential for replacement of lost cells is extremely low. Thus, any intervention that can block this sequence of adverse events as close to the starting point as possible (i.e., the generation of superoxide anion by the electron transport system), should be considered a promising candidate to reduce age-related pathology and delay aging. An instructive line of research would be to elucidate how birds, with their very high metabolic rate, manage this potential oxidative stress problem (Holmes and Austad, 1995). Blocking apoptosis has also partially ameliorated pathological consequences in animal models of neurodegenerative disease and stroke (Friedlander et al., 1997; Kang et al., 2000), although apoptosis may also have positive roles during aging (Warner et al., 1997).

Overlap between biomarkers of aging and indicators of functional status

As defined earlier, biomarkers of aging can be interpreted to mean a parameter or set of parameters that define characteristics related to increasing mortality with chronological age. Another interpretation could relate to a set of parameters that define functional ability (i.e., physiological, cognitive and physical function), and its relationship to morbidity and mortality with chronological age. While the first definition seems best suited for establishing research approaches toward the understanding of the fundamental physiological and metabolic processes of aging, this second definition is applicable to the need of the clinician who manages patients requesting recommendations and/or therapies to reduce their morbidity and extend longevity. It is recognized that both definitions have value when applied in their respective settings, but are likely to converge with one another as the basic mechanisms of aging in humans become better established. It is reasonable to assume that real biomarkers of aging will also correlate with risks for multiple degenerative changes and functional decline in a variety of species.

In the absence of a more complete understanding of the mechanism of aging, clinicians would like to have age-related biomarkers that have adequate predictive value to provide qualified information to their patients to help improve organ-specific function throughout the life cycle, and reduce unnecessary morbidity and premature mortality. These biomarkers might be more than disease risk factors, and represent individual indicators of functional status. Clinicians might prefer a panel of functional biomarkers of aging that relate to health span. In concordance with Dr. Miller's criteria, these biomarkers should:

1. Predict physiological, cognitive and physical function in an age-coherent way, and do so better than chronological age.
2. Predict the years of remaining functionality, and the trajectory toward organ-specific illness in the individual.
3. Be minimally invasive, and accessible to many individuals.

There are several types of data that could constitute a panel of functional biomarkers of aging, including anthropometric data (body mass index, body composition, bone density, etc.), functional challenge tests (glucose tolerance test, forced vital capacity, etc.), physiological tests (cholesterol/HDL, glycosylated hemoglobin, homocysteine, etc.), genomic and proteomic tests.

Such a set of putative functional biomarkers of aging could be measured in a large group of adults who are at an age where functional loss is known to occur most rapidly, such as in the 60 to 70 age group, but it would also be useful to have data on younger adults as well. Statistical evaluation of the data using cluster analysis, pattern recognition, and principal component analysis would help to identify tests that had the greatest predictive value when matched against functional outcome and morbidity patterns. Those with the highest predictive value would be defined as functional biomarkers of aging. These parameters could then be used to test specific clinical approaches and therapies that focus on improvement of physiological, cognitive and physical functioning and their relationship to functional age. The optimal goal would be to obtain a panel of functional biomarkers of aging that could be used to develop personalized medicine or other interventions which effectively reduce morbidity and improve organ-specific function, thereby delaying the necessity for costly hospitalization or social support of the

aging population. At least one such attempt to do this has already been reported (Hochschild, 1990).

Policy implications

How can support be obtained for a biomarker research agenda? The research program supported for 10 years (1988-1998) by the NIA was accomplished through set-aside funds, and use of an ad hoc review process. Review of applications for biomarker research by regular Center for Scientific Review peer review groups at the NIH is not likely to result in enough funded applications to make substantial progress in this area in the near future because of the perceived non-mechanistic character of the research. Clearly, a non-traditional long term source of funding is required, possibly involving commercial or philanthropic sources of support. However, as long as the Food and Drug Administration has no program for evaluating putative anti-aging interventions, commercial organizations are unlikely to perceive sufficient pay off-for funding such aging research.

Some biomarker-relevant research is funded by NIA-funded centers, such as the Nathan Shock Centers, for example, in their gene expression microarray and animal model development cores, but none of these Centers has an overt commitment to biomarker research per se at this time. Moreover, research at these Centers remains more focused on basic mechanisms than on human physiology.

Public education

There are individuals and organizations in the USA who would have us believe that aging is not inevitable, and that “immortality is within our grasp” (Shelton, 2000). These same individuals believe there already exist well-validated biomarkers of aging which can be evaluated at a cost of several thousand dollars per person, and that these evaluations can then be used to design individualized anti-aging treatments. Unfortunately these treatments include some poorly validated interventions such as improving anti-oxidant status and hormone replacement therapies, including growth hormone, testosterone, dehydroepiandrosterone (DHEA), melatonin, etc. Although it is possible that by providing evidence of dysregulation of differentiated cell function, age-related hormonal changes may serve as useful markers of physiological aging, this has not been demonstrated experimentally for either humans or animals. While it is seductive to believe that restoration of hormone levels back to levels that exist in young persons should be a good thing, and although hormone replacement trials have yielded some positive results (at least in the short term), it is clear that negative side effects also may occur, in the form of increased risk for cancer, cardiovascular disease, behavior changes, etc. Estrogen replacement therapy in women has been shown to have definite benefits, especially for prevention of osteoporotic fractures, although some recent studies have raised “red flags” with regard to the usefulness of estrogen for treating or preventing coronary heart disease.The risk/benefit ratios for testosterone replacement and GH treatment have not been established in older persons. Finally, trials of DHEA have failed to show significant clinical benefits in normal aging. Clinical trials to investigate the risks and benefits of these and other potential interventions are either still going on, or have not yet provided definitive answers, and the public is advised to be cautious in requesting these popular anti-aging interventions until adequate clinical trials have been completed and analysed.

As important as reporting promising findings in biomarker research is demonstrating when popular "anti-aging" interventions have no effect, or worse, when they have adverse effects.The majority of participants in this workshop expressed concern about the use of human growth hormone, DHEA, melatonin, various antioxidants and other agents that are claimed to retard or reverse aging, especially given the fact that there are currently no valid biomarkers of human aging. On the other hand, the participants strongly recommended continuing research on these and other hormones, antioxidants and other agents that might have favorable effects upon the promotion of health (e.g., the possibility that some anabolic hormones might protect, if only for a short term, against the frailties of old age).

Concern was expressed over the Dietary Supplement and Health Education Act of 1994. It opened the doors to a multi-billion dollar health food store and Internet business that promotes a variety of agents that are claimed to retard aging and overcome age-related diseases. There is no FDA supervision even to assure the purity of substances offered for sale, let alone their effectiveness and dangers.

The concept of "anti-aging medicine" contrasts with modern gerontology which distinguishes between aging as natural phenomena and diseases, and the role of aging per se as a risk factor for diseases. Anti-aging medicine is not an established specialty although it is being hailed as such. Many lucrative medical practices have emerged which operate outside of the formal insurance system. Systems that suggest they have the ability to measure biomarkers of aging and that they possess the means to favorably affect them are not scientifically-based. These practitioners of anti-aging medicine should be distinguished from mainstream clinicians who are concerned with health promotion and disease prevention.

Nevertheless, advancement of more favorable lifestyles with attention to diet, exercise, tobacco cessation and early identification of risk factors, measurements of functional status and disease markers is a desirable and achievable goal. For example, it is important to lower cholesterol levels through exercise or the use of pharmacological agents like statins, and to detect hypertension and diabetes early in order to effect appropriate control and prevent the often lethal consequences of both. ■

Commentary and Acknowledgement

By Robert N. Butler, M.D. and Richard L. Sprott, Ph.D.

This publication is part of the Canyon Ranch Series, which focuses on bringing to public attention some of the significant ongoing work being done by researchers in a variety of fields to improve the health and longevity of individuals as they grow older. Maintaining Healthy Lifestyles discusses what social and behavioral scientists, and behavioral health experts have learned about helping people control the greatest risk factors facing Americans today: tobacco, a sedentary life and poor diet. Achieving Cognitive Vitality with Aging brings yet another dimension to lifestyle modification, demonstrating that not only physical status, but also mental status is subject to modification.

It must be strongly emphasized that the claims of a "specialty" area of medicine known as "anti-aging medicine" are not rooted in academic medicine and science, and that anti-aging medicine is not a recognized specialty. Further, substances recommended by "anti-aging" doctors, such as DHEA and melatonin (n-acetyl-5-methoxytrypt-amine) have not been demonstrated to either significantly influence the health of older persons or to retard aging processes. Moreover, there can be dangerous side-effects. It should be understood that such notions are distinct from geriatric medicine, a growing and recognized field, designed to promote the health and treat the illnesses of older persons.

The concept of biomarkers remains an extremely important one. The effort to find nonlethal indicators of aging processes is well-defined in the criteria offered in the first part of this workshop report. Utilizing the methodology of caloric restriction in rodent and other animal models is one way to identify and validate such biomarkers if they exist and, indeed, eventually a complex panel of such biomarkers may provide increasing means of assaying the underlying critical age-related biological changes.

Because of the high association of aging with disease, and the importance of discovering biomarkers, additional support for research aimed at defining measures of the biological rate of aging should be made available beyond present funding levels at the National Institutes of Health. Indeed, hardly more than $100 million of the almost $18 billion in the NIH Fiscal Year 2000 budget was devoted to understanding basic processes that are relevant to late life disease, and even less is dedicated to understanding aging itself. While increasing amounts of money must go to understanding the genetic and environmental factors in health and disease, too little is devoted to research into the biological properties of aging.

The ILC-USA thanks Dr. Huber Warner for his remarkable efforts in preparing this report, and in re-creating the atmosphere and the healthy intellectual debate that characterized this extremely energizing workshop.

Thanks also goes to Nora O'Brien, M.A. for her assistance in coordinating the workshop and preparing this document, and to Judith Estrine for editing and supervising the publication of this workshop report.

Special recognition goes to The Ellison Medical Research Foundation, the Kronos Longevity Research Institute and the Institute for the Study of Aging for their intellectual as well as financial contribution to the proceedings.

The ILC-USA also gratefully acknowledges Canyon Ranch Health Resort for their continuous support for the Canyon Ranch-ILC Educational Series. Special recognition goes to Dr. Gary Frost of Canyon Ranch for his role in bringing these Workshops to fruition.

And finally, the man who is a walking symbol for the finest of healthy lifestyles, the ILC-USA pays special tribute to our Board member and founder of Canyon Ranch — Mel Zuckerman — whose generosity and inspiration made this Workshop possible. ■

Literature Cited

Ailion M., Inoue T, Weaver CI, et al. 1999. Neurosecretory control of aging in *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. USA 96: 7394-7397.

Apfeld J, and Kenyon C. 1999. Regulation of lifespan by sensory perception in *Caenorhabditis elegans*. Nature 402: 804-809.

Bartke A, Brown-Borg H, Mattison J, et al. 2001. Prolonged longevity of hypopituitary dwarf mice. Exp. Gerontol. 36: 21-28.

Bookheimer SY, Strojwas MH, Cohen MS, et al. 2000. Patterns of brain activation in people at risk for Alzheimer's disease. N. Engl. J. Med. 343: 450-456.

Brown-Borg HM, Borg KE, Meliska CJ, and Bartke A. 1996. Dwarf mice and the ageing process. Nature 384: 33.

Carney JM, Starke-Reed PE, Oliver CN, et al. 1991. Reversal of age-related increase in brain protein oxidation, decrease in enzyme activity, and loss in temporal and spatial memory by chronic administration of the spin-trapping compound N-tert-butyl-a-phenylnitrone. Proc. Natl. Acad. Sci. USA 88: 3633-3636.

Coschigano KT, Clemmons D, Bellush LL, and Kopchick JJ. 2000. Assessment of growth parameters and life span of GHR/BP gene disrupted mice. Endocrinology 141: 2608-2613.

Dozmorov I, Bartke A, and Miller RA. 2001. Array-based expression analysis of mouse liver genes: Effect of age and of the longevity mutant Prop1^{df}. J. Gerontol. 56A: B72-B80.

Finch C.E. 1990. Longevity, Senescence, and the Genome. Chicago: University of Chicago Press.

Friedlander RM, Brown RH, Gagliardini V, et al. 1997. Inhibition of ICE slows ALS in mice. Nature 388: 31.

Green DR, and Reed JC. 1998. Mitochondria and apoptosis. Science 281: 1309-1312.

Harley CB, Futcher AB, and Greider CW. 1990. Telomeres shorten during ageing of human fibroblasts. Nature 345: 458-460.

Herschman HR, MacLaren DC, Iyer M, et al. 2000. Seeing is believing: Non-invasive, quantitative, and repetitive imaging of reporter gene expression in living animals, using positron emission tomography. J. Neurosci. Res.59: 699-705.

Hochschild R. 1990. Can an index of aging be constructed for evaluating treatments to retard aging rates? A 2,462 person study. J. Gerontol. 45: B187-214.

Holmes DJ, and Austad SN. 1995. Birds as animal models for the comparative biology of aging. J. Gerontol. 50A: B59-66.

Jennings BJ, Ozanne SE, Dorling MW and Hales CN. 1999. Early growth determines longevity in male rats and may be related to telomere shortening in the kidney. FEBS Lett. 448: 4-8.

Kajstura J, Pertoldi B, Leri A, et al. 2000. Telomere shortening is an *in vivo* marker of myocyte replication and aging. Am. J. Pathol. 156: 813-819.

Kang S-J, Wang S, Hara H, et al. 2000. Dual role of caspase-11 in mediating activation of caspase-1 and caspase-3 under pathological conditions. J. Cell Biol. 149: 613-622.

Kimura KD, Tissenbaum HA, Liu Y, and Ruvkun G. 1997. *daf-2* an insulin receptor-life gene that regulates longevity and diapause in *Caenorhabditis elegans*. Science 277: 942-946.

Larsen PL, Albert PS, and Riddle DL. 1995. Genes that regulate both development and longevity in *Caenorhabditis elegans*. Genetics 139: 1567-1583.

Lee C-K, Klopp RG, Weindruch R, and Prolla TA. 1999. Gene expression profile of aging and its retardation by caloric restriction. Science 285: 1390-1393.

Lee C-K, Weindruch R, and Prolla TA. 2000. Gene expression profile of the ageing brain in mice. Nature Genetics 25: 294-297.

Lipman RD, Dallal GE, and Bronson RT. 1999a. Lesion biomarkers of aging in B6C3F1 hybrid mice. J. Gerontol. 54A: B466-477.

Lipman RD, Dallal GE, and Bronson RT. 1999b. Effects of genotype and diet on age-related lesions in *ad libitum* fed and calorie-restricted F344, BN and BNF3F1 rats. J. Gerontol. 54A: B478-491.

Mac Beath G, and Schreiber SL. 2000. Printing proteins as microarrays for high-throughput function determination. Science 289: 1760-1763.

Markowska AL, and Breckler SJ. 1999. Behavioral biomarkers of aging: Illustration of a multivariate approach for detecting age-related behavior changes. J. Gerontol. 54A: B549-566.

Masoro EJ. 1988. Physiological system markers of aging. Exp. Gerontol. 23: 391-394.

Masoro EJ. 2000. Caloric restriction and aging: an update. Exp. Gerontol. 35: 299-305.

Melov S, Ravenscroft J, Malik S, et al. 2000. Extension of life-span with superoxide dismutase/catalase mimetics. Science 289: 1567-1569.

Miller RA. 1997. When will the biology of aging become useful? Future landmarks in biomedical gerontology. J. Am. Geriat. Soc. 45: 1258-1267.

Miller RA. 1999. Kleemeier Award Lecture: Are there genes for aging? J. Gerontol. 54A: B297-307.

Miller RA, Chrisp C, and Atchley W. 2000. Differential longevity in mouse stocks selected for early life growth trajectory. J. Gerontol. 55A: B455-461.

Miller RA, Galecki A, and Shmookler-Reis RJ. 2001. Interpretation, design and analysis of gene array expression experiments. J. Gerontol. 56A: B52-B57.

Oshima J. 2000.The Werner syndrome protein: an update. Bioessays 22: 894-901.

Parkes TL, Elia AJ, Dickinson D, et al. 1998. Extension of *Drosophila* lifespan by overexpreesion of human SOD1 in motorneurons. Nature Genetics 19: 171-174.

Perls T, Shea-Drinkwater M, Bowen-Flynn J, et al. 2000. Exceptional familial clustering for extreme longevity in humans. J. Am. Ger. Soc. 48: 1483-1485.

Perls T. 2001. Genetic and phenotypic markers among centenarians. J. Gerontol. 56A: M67-M70.

Pero RW, Hoppe C, and Sheng Y. 2000. Serum thiols as a surrogate estimate of DNA repair correlates to mammalian life span. J. Anti-aging Med. 3: 241-249.

Rimm EB, Stampfer MJ, Ascherio A, et al. 1993. Vitamin E consumption and risk of coronary heart disease in men. N. Engl. J. Med. 328: 1450-1456.

Rogina B, Reenan RA, Nilsen SP, and Helfand SL. 2000. Extended life-span conferred by cotransporter gene mutations in *Drosophila*. Science 290: 2137-2140.

Shay JW, Wright WE. 2001. When do telomeres matter? Science 291: 839-940.

Sheldon D, Dipping into the fountain of youth. American Medical News, 12/4/2000.

Small GW, Ercoli LM, Silverman DH, et al. 2000. Cerebral metabolic and cognitive decline in persons at genetic risk for Alzheimer's disease. Proc. Natl. Acad. Sci. USA 97: 6037-6042.

Sonntag WE, Lynch CD, Cefalu WT, et al. 1999. Pleiotropic effects of growth hormone and insulin-like growth factor (IGF-1) on biological aging: Inferences from moderate caloric-restricted animals. J. Gerontol. 54A: B521-538.

Sprott RL. 1988. Preface to special issue on biomarkers of aging. Experimental Gerontology 23: iii

Sprott RL. 1999. Biomarkers of Aging. J. Gerontol. 54A: B464-465.

Stampfer MJ, Hennekens CH, Manson JE, et al. 1993. Vitamin E consumption and risk of coronary heart disease in women. N. Engl. J. Med. 328: 1444-1449.

Sun J, and Tower J. 1999. FLP recombinase-mediated induction of Cu/Zn-superoxide dismutase transgene expression can extend life span of adult *Drosophila melanogaster* flies. Mol. Cell Biol. 19: 216-228.

Turturro A, Witt WW, Lewis S, et al. 1999. Growth curves and survival characteristics of the animals used in the biomarkers of aging program. J. Gerontol. 54A: B492-501.

Warner HR, Hodes RJ, and Pocinki K. 1997. What does cell death have to do with aging? J. Am. Geriat. Soc. 45: 1140-1146.

Warner HR, Ingram D, Miller RA, et al. 2000. Program for testing biological interventions to promote healthy aging. Mech. Ageing Develop. 115: 199-208.

Wolf NS, and Pendergrass WR. 1999.The relationships of animal age and caloric intake to cellular replication *in vivo* and *in vitro*: A review. J. Gerontol. 54A: B502-517.

Wolkow CA, Kimura KD, Lee M-S, and Ruvkun G. 2000. Regulation of *C. elegans* life-span by insulin like signaling in the nervous system. Science 290: 147-150.

Yan L-J, Levine RL, and Sohal RS. 1997. Oxidative damage during aging targets mitochondrial aconitase. Proc. Natl. Acad. Sci. USA 94: 11168-11172.

Yan L-J, and Sohal RS. 1998. Mitochondrial adenine nucleotide translocase is modified oxidatively during aging. Proc. Natl. Acad. Sci. USA 95: 12896-12901.

Zhen X, Uryu K, Cai G, et al. 1999. Age-associated impairment in brain MAPK signal pathways and the effect of caloric restriction in Fischer 344 rats. J. Gerontol. 54A: B539-548.

Glossary

Adenine Nuclotide Translocase – an enzyme required for transport of ATP from mitochondria to the cytoplasm.

Alzheimer's Disease – an aging-dependent disease characterized by loss of memory. Risk factors include both genetic and environmental factors. Age of onset varies from the late 40s for patients with early-onset genetic risk factors, to 65 and older for most other patients.

Aminoguanidine – a compound used to reduce non-enzymatic glycosylation of proteins.

Antioxidant – a compound and/or enzyme which neutralizes reactive oxygen species, thereby reducing oxidative stress.

Apoptosis – a genetically regulated program leading to cell suicide.

ATP – adenosine triphosphate, the major form of biological energy present in cells.

Biomarker (of Aging) – an age-related change which reflects the physiological age of an individual, in contrast to the chronological age.

Caloric Restriction – a diet strategy to limit the caloric intake, while supplying all other essential dietary ingredients. This extends life expectancy and delays the onset of age-related disease in rodents.

Caenorhabditis Elegans (C. elegans) – Latin name for species of nematode, a small soil-dwelling worm, which has been developed for biomedical research because of its well characterized developmental program; it is a useful model system for studying aging because of its short life span.

CIS-Aconitase – an enzyme involved in the citric acid cycle.

Citric Acid Cycle – a biochemical pathway found primarily in mitochondria, which provides energy for ATP synthesis.

Coenzyme Q – a component of the electron transport system in mitochondria.

Cytochrome C – a mitochondrial protein required for ATP synthesis; leakage of this protein into the cytoplasm induces apoptosis.

daf – a symbol for C. elegans mutants with developmental defects.

DNA Helicase – an enzyme which promotes separation of two complementary strands of DNA.

Dehydroepiandrosterone (DHEA) – a circulating adrenal steroid hormone which has been widely promoted as an "anti-aging" hormone; circulating levels decrease with age.

Eukaryotic – organisms whose DNA is present as chromosome pairs (includes all plants and animals).

Exonuclease – an enzyme which degrades DNA one nucleotide at a time; such enzymes are involved in DNA replication, repair, and recombination.

Glutamine Synthetase – an enzyme which combines ammonia with glutamate, both of which are neurotoxic, to form glutamine, which is non-toxic.

Growth Hormone – a hormone produced in the pituitary which is essential for normal growth; circulating levels decrease with age, and growth hormone replacement has been promoted as a possible "anti-aging" intervention.

8-Hydroxyguanine – one of many damaged bases found in DNA as a result of oxidative stress.

Life Expectancy – the average number of remaining years an individual can expect to live at any given age.

a-lipoic Acid – a simple organic molecule containing a sulfhydryl group; thus it is an antioxidant.

Longevity – the length of life of an individual, or the average length of life of a population of individuals.

MAPK – mitogen-activated protein kinase, a protein involved in signal transduction.

Maximum Life Span – the longest observed life span of an individual of any species.

Melatonin – a hormone produced in the pineal gland, which has a role in the sleep/wake cycle; the circulating level of melatonin decreases with age and melatonin replacement has been promoted as a possible "anti-aging" intervention.

Microarry Technology – technology permitting the assay of thousands of samples simultaneously without individual handling.

Mitochondria – an organelle within the cell where glucose and oxygen are metabolized to produce ATP, the main cellular source of biological energy.

N-acetyl Cysteine – a simple organic molecule containing a sulfhydryl group; thus it is an antioxidant.

Oxidative Stress – the process whereby cellular macromolecules are damaged by reactive oxygen species, produced mainly in the mitochondria, leading to dysfunction.

Pituitary – the gland which produces several hormones, including growth hormone.

Pply ADP-ribose Polymerase – an enzyme involved in DNA repair.

Prolactin – a hormone produced in the pituitary gland, acting primarily on the mammary gland to promote lactation.

Reporter Gene – a gene whose expression is easily measured, often because it produces a colored product.

Signal Transduction – the process of relaying a biological signal from one part of a cell to another.

Single Nucleotide Polymorphism (SNP) – a sequence difference in DNA found with relatively high frequency within a population.

Sulfhydryl Group – a sulfur-containing group with antioxidant properties found in some biological molecules.

Superoxide Dismutase (SOD) – an anti-oxidant enzyme which converts the superoxide anion to hydrogen peroxide.

T-lympocyte – a white blood cell produced by the thymus.

Thyroid Stimulating Hormone – a hormone produced in the pituitary gland which stimulates growth of the thyroid.

Transcription – the process of copying the sequence of DNA into messenger RNA.

Transgene – a gene from one organism inserted into another.

unc – a symbol for C. elegans mutants which appear to be "uncoordinated".

Werner's Syndrome – a genetic disease characterized by premature development of adverse age-related changes such as cataracts, cardiovascular disease, cancer; cataracts may develop as early as the 20s, with average age of death at 45-50 years.

INTERNATIONAL LONGEVITY CENTER–USA

Board of Directors

INTERNATIONAL LONGEVITY CENTER INTERNATIONAL CENTERS

Directors

The International Longevity Center–USA (ILC-USA) *is a not-for–profit,non-partisan research, education and policy organization whose mission is to help individuals and societies address longevity and population aging in positive and productive ways and highlight older people's productivity and contributions to their families and society as a whole.*

The organization is part of a multinational research and education consortium, which includes centers in the U.S., Japan, Great Britain, France and the Dominican Republic.These centers work both autonomously and collaboratively to study how greater life expectancy and increased proportions of older people impact nations around the world.

Single copies are free.
Multiple copies are $3.00 each.

NOTES

THE INTERNATIONAL LONGEVITY CENTER–CANYON RANCH SERIES

Prescription for Longevity: Fads and Reality

Maintaining Healthy Lifestyles: A Lifetime of Choices

Achieving and Maintaining Cognitive Vitality with Aging

Biomarkers of Aging: From Primitive Organisms to Mans

COMING SOON

Anti-Aging Medicine

Longevity Genes: From Primitive Organisms to Man

Biomarkers of Aging: From Primitive Organisms to Man

INTERNATIONAL LONGEVITY CENTER–USA

60 East 86th Street
New York, NY 10028
212 288 1468 Tel
212 288 3132 Fax
www.ilcusa.org

An Affiliate of the Mount Sinai School of Medicine