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Biochemical Markers of Aging for Longitudinal Studies in Humans

Peter M. Engelfriet*, Eugène H. J. M. Jansen, H. Susan J. Picavet, and Martijn E. T. Dollé

* Correspondence to Dr. Peter M. Engelfriet, National Institute for Public Health and the Environment (RIVM), Centre for Prevention and Health Services Research, P.O. Box 1, 3720 BA Bilthoven, The Netherlands (e-mail: peter.engelfriet@rivm.nl).

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Much progress has been made in the past decades in unraveling the mechanisms that are responsible for aging. The discovery that particular gene mutations in experimental species such as yeast, flies, and nematodes are associated with longevity has led to many important insights into pathways that regulate aging processes. However, extrapolating laboratory findings in experimental species to knowledge that is valid for the complexity of human physiology remains a major challenge. Apart from the restricted experimental possibilities, studying aging in humans is further complicated by the development of various age-related diseases. The availability of a set of biomarkers that really reflect underlying aging processes would be of much value in disentangling age-associated pathology from specific aging mechanisms. In this review, we survey the literature to identify promising biochemical markers of aging, with a particular focus on using them in longitudinal studies of aging in humans that entail repeated measurements on easily obtainable material, such as blood samples. Our search strategy was a 2-pronged approach, one focused on general mechanisms of aging and one including studies on clinical biomarkers of age-related diseases.

aging; biomarkers; longitudinal studies

Abbreviation: ROS, reactive oxygen species.

INTRODUCTION

In studying how we age and why people age at different rates, the availability of suitable biomarkers is highly desirable. Ideally, such biomarkers should reflect underlying mechanisms of aging and thus make it possible to distinguish essential aging phenomena from processes that are due to the various diseases that often occur with advancing age. Some of these diseases actually might be part of the aging process itself and represent forms of "accelerated aging," such as seen in progeroid syndromes (1). However, it remains a major challenge to distinguish potentially avoidable disease processes from underlying "true" aging. Currently, no broad consensus exists on how to draw the line between aging and age-related disease mechanisms (2–4). Nevertheless, in 1962, B. Strehler (5) proposed 5 criteria to define aging that still could serve as a guide in deciding whether a proposed biomarker might be an indicator of aging: Changes seen with advancing age should be cumulative, progressive, intrinsic, deleterious to biologic function, and universal (i.e., observed in all members of a species) (5). In validating whether identified markers indeed gauge aging mechanisms, longitudinal studies, in which biomarkers are measured repeatedly over time in the same human subjects, seem to be especially rewarding.

In the present review, we intend to provide an overview of the biomarkers of aging, both those that are currently known and those that seem to be promising candidates. We will take a broad view and consider biomarkers from 2 different angles. On the one hand, we will draw from the literature on more basic aging studies conducted in the laboratory, hoping to find markers for specific aging processes. On the other hand, we will take our lead from the wealth of epidemiologic and clinical studies on biomarkers of strongly age-related diseases. Although markers for such diseases are used mostly for different purposes, such as diagnosis or prognosis, we speculate that some are also directly related to aging. We focus on biochemical markers that can be measured in blood, urine, saliva, or easily obtainable tissues, such as buccal cells and peripheral blood lymphocytes.

METHODS

Currently, there is no universally accepted definition of a biomarker of aging (4). We have not attempted to provide one of our own. Rather, because this is an exploratory review, we have accepted the senses in which the term is used in the literature.

In searching the literature, a 2-tiered approach was followed. First, general reviews and textbooks were consulted in a broad manner to define more focused and specific search terms. For general mechanisms of aging, we consulted the latest edition of the well-known textbook edited by Masoro and Austad (6), as well as general reviews published in the major biomedical journals. On the basis of these, we defined more specific terms and phrases, such as "sirtuins AND aging." We limited the search to studies in humans indexed in PubMed.

For biomarkers of (age-related) diseases, we took a similar approach. On the basis of general reviews on biomarkers for specific diseases, using search terms such as "cardiovascular disease AND (biomarkers OR disease marker)" or "lung emphysema AND biomarkers," we looked for specific markers that were further investigated for a relation to aging. Examples are "interleukin-6 AND (age OR aging)" and "natriuretic peptides AND (age OR aging)."

Because dysfunction of a particular organ might or might not be due to aging mechanisms, a strategy was formulated to decide which biomarkers were relevant to aging. Although 4 of the 5 criteria proposed by Strehler (5) (described in the Introduction of the present review) could apply to both disease and aging, the criterion that the changes should be universal does offer a principle to distinguish organ system aging from disease-related dysfunction (5). Thus, ideally, the sought biomarkers should be specific for changes that occur in a particular organ in virtually all humans as they age—for instance, an increase in the "stiffness" of the blood vessels.

An overview of the biomarkers mentioned in the present review is given in the tables. To render the review more useful for researchers, we have supplemented the tables with 2 types of information. First, we provide details about the laboratory tests that are in use for determining the reviewed biomarkers. Second, the tables contain a column providing references to epidemiologic studies where relevant and available. However, a detailed and comprehensive discussion of such studies and the methodological issues involved is beyond the scope of this review.

RESULTS

General mechanisms of aging

The "young" science of aging has made remarkable progress since the discovery in the 1980s that specific gene mutations in yeast, worms, and flies can extend their lifespan (6). Evolutionarily conserved signaling pathways that regulate growth and reproduction, energy metabolism, and nutrient sensing are involved in longevity (7). Although the road from small, short-lived organisms to the organizational complexity of the human body is not straightforward, laboratory studies of investigational species have provided many keys to the search for general markers of aging mechanisms in humans. In general, these mechanisms take place within the cells, in subcellular compartments, and tissue samples are required for their investigation (8). As mentioned previously, we focus on biochemical markers that can be assessed in biomaterial such as blood.

Aging cells and tissue remodeling. In most tissues, a proportion of the cells die and are replaced over time. Cells are replaced from a pool of stem cells. When stem cells lose their capacity to replicate and differentiate, becoming senescent cells, the number of functional cells will decrease, which leads to loss of homeostasis and ultimately to tissue dysfunction. Two types of cell senescence are recognized: replicative senescence and stress-induced premature senescence (9, 10). It has been proposed that the length of telomeres could be a measure for replicative senescence. This is discussed further in the next section. Stress-induced premature senescence refers to the concept that stressful stimuli such as oxidative stress or DNA damage can accelerate senescence. Such stimuli could bring cells to the point at which apoptosis starts or at which activation of the p53/p21 pathway leads to permanent cell cycle arrest (9). Despite its unfavorable connotation, cell senescence seems to be a protective mechanism against malignant transformation at first. However, as senescent cells accumulate, they can actively contribute to tissue dysfunction by creating deregulating microenvironments (11). In the past few years, upregulation of caveolin-1 expression has been identified as an important mediator of cell senescence (10). This molecule can be studied in cells grown in culture and in blood (12). Analysis of blood samples offers an interesting "window" on the availability of stem cells of certain types, which, theoretically, could change with age. Various types of stem cells can be detected and quantified in blood—for instance, endothelial progenitor cells and other blood stem cells (13–15).

Many age-related observable changes, such as cardiac remodeling and increasing arterial stiffness, involve changes in the extracellular matrix, the tissue in which the cells reside. For example, when stimulated by growth factors, cardiac fibroblasts synthesize and secrete matrix proteins such as collagen and tissue inhibitor of matrix metalloproteinases, whereas proinflammatory mediators stimulate the secretion of matrix metalloproteinases (16). Higher levels in blood probably represent more active "tissue remodeling." Table 1 mentions a few markers of extracellular matrix remodeling that can be measured in blood and provides references to human studies.

Telomeres. Replicative senescence is associated with what has become known as the Hayflick limit: the restricted ability of cells in culture to continue dividing (17). It depends on the number of divisions a cell has undergone, for which the length of telomeres has been proposed as a measure. The telomere is a specialized structure located at the end of chromosomes. While replicating the genome before cell division, DNA polymerase cannot fully replicate the 3′ end of the chromosome DNA strand. But for the presence of telomerase, part of the telomere would be lost, and

Table 1. Biomarkers Identified From Studies of General Mechanisms of Aging

Underlying Biologic Process	Possible Biomarker	Epidemiologic Studies (References)	Level of Validation[a]	Sample Type	Analytic Method	Throughput (High/Low)[b]	Further References
Aging cells and tissue remodeling	Caveolin-1	Mainly studied in cultured cells. Significance in plasma unclear.	3	Serum/plasma	Enzyme immunoassay	High	(10, 166, 167)
	Endothelial progenitor cells; blood stem cells	(111)	3	Fresh anticoagulated blood	Flow cytometric assay	Low	(13–15, 110, 112)
	Extracellular matrix remodeling: matrix metalloproteinase-9, tissue inhibitor of metalloproteinase-1, galectin-3	(168, 169) (clinical populations)	2	Serum/plasma	Enzyme immunoassay	High	(16, 170)
Telomeres and telomerase	Leukocyte telomere length	(171)	1	Blood lymphocytes	Quantitative PCR; terminal restriction fragment Southern blotting, quantitative fluorescence in situ hybridization	Low to high	(19, 23, 24)
	Telomerase RNA component; telomerase reverse transcriptase		1	Blood lymphocytes	Telomeric repeat amplification protocol followed by enzyme immunoassay or fluorescent product quantification	Low to medium	(19, 32)
DNA repair	8-Hydroxydeoxyguanosine	(34)	3	Serum/plasma/urine	Enzyme immunoassay	Medium	(35)
	Circulating anti-DNA antibodies		3	Serum/plasma	Enzyme immunoassay	High	(36)
Growth, energy, and reproductive function	Sirtuin-1, sirtuin-2			Blood lymphocytes	Enzyme immunoassay	High	
	Growth hormone, insulin-like growth factor 1	(172, 173) (174) (review)	1	Serum/plasma	Enzyme immunoassay	High	(45, 46)
	Insulin, glucose	(35, 49) (174) (review)		Serum/plasma	Autoanalyzer	High	
	Glycosated hemoglobin ($HbA1_c$)	(35, 76, 174) (reviews)		Erythrocytes	Autoanalyzer	High	
	Pentosidine			Serum/plasma	HPLC	Medium	
	Other advanced glycation end products		2	Serum/plasma	Enzyme immunoassay/ HPLC	Medium	(175)
	Receptor for advanced glycation end products			Serum/plasma	Enzyme immunoassay	High	
	Luteinizing hormone, follicle-stimulating hormone, dehydroepiandrosterone	(62–63, 176) (174) (review)	1	Serum/plasma	Autoanalyzer	High	(61)

Table continues

Table 1. Continued

Underlying Biologic Process	Possible Biomarker	Epidemiologic Studies (References)	Level of Validation[a]	Sample Type	Analytic Method	Throughput (High/Low)[b]	Further References
	Triiodothyronine		2	Serum/plasma	Autoanalyzer	High	(45)
	Cortisol	(64) (76, 174) (reviews)	3	Serum/plasma	Autoanalyzer	High	
	Leptin, adiponectin	(177, 178) (174) (review)	2	Serum/plasma	Enzyme immunoassay	High	(179)
Protein metabolism	Serum amyloid A (acute-phase protein, associated with inflammatory responses)	(180) (clinical population)	3	Serum	Enzyme immunoassay	High	
	Nicotinamide phosphoribosyltransferase (visfatin)		2	Serum/plasma	Enzyme immunoassay	High	(41)
	Carbonyls, Michael adducts	(35) (review)	2	Serum/plasma	Enzyme immunoassay	High	
	Eotaxin		4	Serum/plasma	Enzyme immunoassay	High	(75)
	Serum N-glycan profile	(181)	1	Serum/plasma	Capillary array electrophoresis	High	
Lipid metabolism	Free fatty acids			Serum/plasma	Autoanalyzer	High	
	Lipid profile: very low-density lipoprotein, low-density lipoprotein, high-density lipoprotein, total cholesterol, triglycerides	(182) (76, 174) (reviews), and numerous other studies	1	Serum/plasma	Autoanalyzer	High	(183)
	Isoprostanes	(35, 174) (reviews)	2	Serum/plasma	Enzyme immunoassay	High	
	Lipid peroxidation products: malondialdehyde, 4-OH-2-nonenal	(184)	2	Serum/plasma	HPLC	Medium	(185, 186)
Oxidative stress	Oxidized low-density lipoprotein			Serum/plasma	Enzyme immunoassay	High	
	Gluthatione, glutathione reductase, glutathione peroxidase	(85) (86) (clinical population) (87) (review)	2	Erythrocytes	Autoanalyzer	Medium	(187, 188)
	Thioredoxin reductase-1			Cells	Autoanalyzer	Medium	
	Superoxide dismutase		3	Erythrocytes	Autoanalyzer	Medium	
	Nitric oxide			Serum/plasma	Autoanalyzer		
	Vitamins A and E		1	Serum/plasma	HPLC		
	Vitamin C		1	Serum/plasma	Autoanalyzer		
	Hypoxia-inducible factor 1		3	Nuclear extract	DNA-bound transcription factor enzyme immunoassay	Medium	(91)

Table continues

Table 1. Continued

Underlying Biologic Process	Possible Biomarker	Epidemiologic Studies (References)	Level of Validation[a]	Sample Type	Analytic Method	Throughput (High/Low)[b]	Further References
Inflammation	Interleukin-6, tumor necrosis factor α, and other "acute-phase proteins"	(88, 189–192) (76, 174) (reviews)	1	Serum/plasma	Enzyme immunoassay	High	
	C-reactive protein	(174) (review)	1	Serum/plasma	Autoanalyzer	High	
	Pentraxin-3			Serum/plasma	Enzyme immunoassay	High	
	Cathepsin S (has a role in major histocompatibility complex class II antigen presentation)	(193)	2	Cathepsin S	Enzyme immunoassay/ enzymatic activity	High	

Abbreviation: HPLC, high-performance liquid chromatography.

[a] The degree to which the mentioned biomarkers may be more or less considered validated with regard to human aging is indicated as follows: 1, widely studied in humans; 2, has been studied in humans with regard to aging; 3, theoretically interesting, and blood or urine tests exist; and 4, theoretically interesting or identified as important in animal models.

[b] Throughput: low: <50 samples/day; medium: 60–80 samples/day; high: >100 samples/day.

chromosomes would become shorter at each replication (18). The protein-RNA telomerase complex prevents this shortening. However, the telomere is also under attack from surrounding factors, such as reactive oxygen species (ROS) (19, 20). In addition, because the abundance of telomerase varies, telomeres ultimately are destined to become shorter (21). The length of telomeres (e.g., leukocyte telomere length) has attracted much attention in recent years and has been studied in the context of various diseases. Reduced leukocyte telomere length has been found to be associated with age-related disease and to be predictive of death (19, 22–24). Because shortening of leukocyte telomere length is under strong environmental influences, particularly damage due to ROS, it is considered by some to be a measure for the cumulative burden of oxidative stress over a lifetime. However, leukocyte telomere length varies considerably between individuals of the same age, even at young ages, and reports relating life expectancy to telomere length have yielded conflicting results (25). Moreover, at present there is much discussion of how to measure leukocyte telomere length accurately, reproducibly, and affordably (23).

The main application of telomere length in epidemiologic studies has been as a measure of (cumulative) oxidative stress, presumed to be associated with chronic diseases (26). Alternatively, others have used it as a prognostic marker and related it to the incidence of morbidity or mortality (27–31). Also, telomerase activity has been studied in relation to age-related diseases and in relation to the presence of major risk factors for cardiovascular disease (19, 32).

DNA repair. Early in the era of DNA studies, it was realized that mechanisms must exist that protect DNA from damaging influences from both endogenous and exogenous sources, such as ROS or ultraviolet radiation. The importance of these mechanisms became increasingly apparent with the discovery that some of the "accelerated aging," or progeroid, syndromes are caused by mutations in genes for proteins involved in DNA repair (33). Since then, it has become clear that the stability of the genome is maintained by a very intricate system of DNA repair and "checkpoint pathways" (33). Most tests that assess DNA repair are indirect: The adequacy of the repair mechanisms is inferred from the observed degree of DNA damage, such as that due to oxidative stress (discussed later). One frequently used marker of oxidative damage of nucleic acids is the 8-hydroxy-2′-deoxyguanosine adduct, formed when the hydroxyl radical acts on deoxyguanine. It can be measured in cells but also in urine, and it has, for instance, been found to be increased in smokers (34, 35). In another test of damaged DNA, the binding of circulating antibodies to DNA that has been damaged by ROS is used (36). So far, markers of DNA damage have not been studied extensively in population studies.

Growth, energy homeostasis, and reproductive function. The mechanisms influencing life span in worms, flies, and yeast involve molecules that sense nutrient status and regulate replication, growth, and energy balance. A signaling pathway centered on the receptor for insulin and insulin-like growth factor 1 emerged as one of the central mechanisms. Unicellular species have a single receptor, whereas humans have 2 separate receptors. This implies greater

complexity of insulin/insulin-like growth factor 1 signaling in humans (37).

In addition, the discovery of the genes coding for the so-called sirtuins as "longevity genes" was one of the milestones in aging research (38). The sirtuins are a class of molecules believed to be essential in mediating the effects of stressful stimuli, such as nutrient scarcity (caloric restriction). (39). They act as protein deacetylases, modifying signaling components, transcription factors, and histones; as such, they influence metabolism, cell division, differentiation, survival, and senescence. The reactions they catalyze depend on nicotinamide adenine dinucleotide and the ratio of the oxidized and reduced forms of nicotinamide adenine dinucleotide. Even though their role as bona fide antiaging proteins has not been universally confirmed, it has become clear that the sirtuins have a central role in various age-related processes. Moreover, in rodent models they have emerged as potent protectors against age-related diseases such as atherosclerosis, diabetes, and cancer (40). This potential has also led to the development of new drugs that influence sirtuin activity (39). Hence, biomarkers of their activity that can be measured in humans would be of great benefit, but so far candidates are lacking. At least one product involved in sirtuin metabolism is found in serum—namely nicotinamide phosphoribosyltransferase (visfatin) (41). Moreover, sirtuins (sirtuin-1 and sirtuin-2) can be assessed in lymphocytes.

In contrast, insulin signaling has been the subject of much clinical research through its causative role in diabetes mellitus, one of the most common age-related diseases. Insulin/insulin-like growth factor 1 signaling forms the core of the so-called somatotropic axis, involving the hypothalamus, the pituitary gland (hypophysis), and the peripheral targets of their secreted hormones. In mammals, the somatotropic axis in turn forms part of the very intricate endocrine system referred to as the hypothalamic pituitary axis, which regulates energy metabolism, growth, reproduction, and reaction to stress (42). Its core is the anterior lobe of the hypophysis, the specialized cells of which synthesize hormones that are secreted into the blood: growth hormone, thyroid-stimulating hormone (43), adrenocorticotropic hormone, prolactin, luteinizing hormone, and follicle-stimulating hormone (44). Their production and secretion are stimulated or inhibited by releasing factors secreted in the hypothalamus that reach the hypophysis via the hypophysial portal circulation. The hormones secreted by the hypophysis in turn stimulate the release of other hormones: Growth hormone stimulates the release of insulin-like growth factor 1, mainly in the liver, and affects insulin secretion and sensitivity for insulin; thyroid-stimulating hormone stimulates the release of triiodothyroxine and thyroxine by the thyroid; adrenocorticotropic hormone is required for the production of glucocorticoids (cortisol) and aldosterone by the adrenal gland; and luteinizing hormone and follicle-stimulating hormone have the gonads as their targets, where they mediate the production of estrogen, progesterone, testosterone, and dehydroepiandrosterone.

The relation of some components of the hypothalamic pituitary axis with human aging has been studied relatively often. The decline of growth hormone and insulin-like growth factor 1 with age has been well documented, and growth hormone supplementation and insulin-like growth factor 1 supplementation have been considered an "anti-aging" treatment (45, 46). However, the role of these 2 hormones in aging remains controversial, in particular whether reduced or increased levels are beneficial. Their relation with risk of death appears to be U shaped, which might be explained by the possibility that increased levels are beneficial for the vascular system but confer an increased risk of cancer (47).

By far most of the epidemiologic studies have examined the key molecules of diabetes, insulin and its target glucose, which are at the peripheral end of the somatotropic axis. On average, fasting glucose levels rise with age—the "progressive glucosemia of normal aging." Even in people without diabetes, a linear increase in glucose levels occurs with age (48). However, in the Framingham cohort, a subset of individuals seemed to escape this steady increase (49). The deleterious effects of increased blood glucose levels are due partly to the chemical reactions between glucose and other molecules, such as amino acid side chains of proteins. The resulting products are advanced glycation end products, such as pentosidine and carboxymethyllysine, which can be detected in serum, as can the secreted form of their receptor, soluble receptor for advanced glycation end products. Glycated hemoglobin is the prototypical example of an advanced glycation end product, which is used together with fructosamine as a long-term biomarker of the glucose status of the body. This parameter also has been found to increase linearly with age (50). It is already an old theory that advanced glycation end product formation is a possible cause of aging (51). Advanced glycation end product formation by glucose and fructose can result in cross-linking of proteins. Cross-linking of collagen in the vessel wall contributes to the stiffening of the blood vessels seen with aging.

The gradual increase in fasting glucose levels with age is believed to be the result of 2 mechanisms involving insulin, the main regulator of blood glucose (52). First, the islets of Langerhans in the pancreas decline in volume, number, and function. The β cells of these islets combat an increase in blood glucose by secreting insulin. Second, peripheral tissues become increasingly insensitive to the action of insulin, which is required for the uptake of glucose by cells such as myocytes. Under normal conditions, β cells can compensate for decreasing insulin sensitivity by increasing their production of insulin.

Because of the complexity of the regulation of glucose and insulin levels, fasting levels of glucose and insulin provide only fairly rough information. Studying the dynamics of insulin secretion in response to a dose of glucose, such as in the oral glucose tolerance test, gives more insight into the insulin secretion response (β-cell function) and its decline with age (52–54). Unfortunately, this requires the drawing of consecutive blood samples in which both glucose and insulin levels are then measured as a function of time. Furthermore, the changes in insulin levels in response to a glucose dose are still difficult to interpret, as the shape of the 2 graphs (glucose and insulin concentrations versus time) is determined by both β-cell function and

insulin sensitivity; in addition, the measured values of insulin are influenced by the degree of hepatic insulin extraction. Because failing β-cell function is believed to be the main cause of diabetes mellitus, sophisticated methods have been developed to obtain a "pure" estimate of β-cell function. Invariably, these involve some form of mathematical modeling (53). One of the concepts that has emerged is the so-called disposition index, the product of β-cell function and insulin sensitivity (52, 55). This measure has been found to decrease with age (56).

Whereas β-cell function is the center of attention when early diagnosis of diabetes mellitus or the risk of diabetes mellitus is at stake, from the point of view of the study of aging, the gradual decrease of insulin sensitivity with age is equally relevant. Because obesity is known to be associated with decreased insulin sensitivity, it is especially relevant to consider age-related changes in body composition, particularly the amount and distribution of fat. Total body weight increases until middle age and then starts to decline slowly (57) because of a loss of fat-free mass, mostly in muscle and bone. Visceral fat increases, but subcutaneous fat decreases. Among the important findings of the past decade is the insight that far from being a passive storage place for energy, fat tissue is metabolically active. Among other functions, it produces hormone-like substances, the adipokines, particularly leptin and adiponectin (58). Their levels are influenced by the aforementioned changes in body composition. It has become clear that the adipokines exert a wide range of effects that are relevant to aging and to age-related diseases and also influence insulin sensitivity. A recent study of a subgroup of the Framingham cohort showed that plasma leptin concentrations are inversely related to the incidence of diseases such as Alzheimer's disease (59, 60).

Reproductive and sexual function also is regulated by the hypothalamic pituitary axis. Decline of sexual function has been considered an exemplary sign of aging probably as long as history has been recorded. Levels of sex hormones decrease with age. It is an open question to what extent these changes have a causative role or whether they merely reflect the aging process. In recent years, dehydroepiandrosterone has received considerable attention with regard to the aging process. Dehydroepiandrosterone and dehydroepiandrosterone sulphate are the sex hormones with the highest concentration in blood. They peak at age 25–30 years and then start to decline to reach approximately 10%–20% of the peak level at 80 years of age (61). The dehydroepiandrosterone sulphate level has been found to be related inversely to death in men more than 50 years of age (62). Associations with risk of death also have been observed in women (63).

Finally, the corticotroph "branch" of the hypothalamic pituitary axis has been studied extensively, especially with regard to responses to stress and much less with regard to aging. Cortisol levels, for example, are seen as modulators of age-related phenomena such as cognitive decline rather than as markers of aging itself (64, 65).

In summary, the physiology of growth and energy homeostasis justifiably occupies the center stage in aging research. The observation that the life spans of yeast and rodents can be extended by dietary restriction has pointed to a crucial role of pathways involved in energy balance (66). Elucidation of the molecular machinery involved has spurred the major translational effort to investigate what determines life span in humans and continues to constitute a challenging research program. Furthermore, interest in this field has received much impetus from the threat to public health posed by the increasing prevalence of obesity, diabetes, and physical inactivity. Therefore, we might expect that much research effort will continue to be devoted to the intricate regulation of glucose and insulin and their varied effects, as well as the metabolically active role of fat tissue and the wide range of effects of the molecules it produces. Additionally, the effects of interventions such as weight reduction, increasing physical activity, and potential new drugs will require assessment of energy metabolism. This system is relatively easily accessible, given that it can be studied largely in blood samples with the use of high-throughput methods.

Proteins and protein metabolism. Proteins are being modified continuously within cells, and they are subject to an elaborate system of "quality control" (67, 68). This entails protein folding, posttranslational modifications (glycosylation, oxidation, nitrosylation), surveillance, repair and removal, and characteristic responses to stress. Alterations in protein homeostasis are common in most tissues in older organisms (69, 70). Also, changes in subsets of chaperones (involved in protein trafficking) have been found in almost all species and tissues (71). In centenarians, an attenuation of the chaperone response has been reported (72–74). When the amount of unfolded proteins in the endoplasmic reticulum passes a certain threshold, the unfolded protein response is triggered (71).

An important source of intracellular stress, also affecting proteins, is the presence of excessive amounts of oxygen radicals (discussed later in "Wear and tear: oxygen metabolism and inflammation"). Markers of oxidative stress can be found, for instance, in the oxidation of proteins. Protein carbonyls can result from the oxidation of the side chains of lysine, arginine, proline, or threonine. An accumulation of carbonyls in plasma has been found in various age-related diseases (35). In turn, carbonyls can give rise to advanced glycation end products, mentioned previously. In addition, proteins can react with products of lipid peroxidation (see "Lipids and lipid metabolism"). Currently, large-scale analysis methods under the name of "proteomics" are rapidly changing the playing field. An interesting successful example of such an approach is the identification of a chemokine, chemokine (C-C motive) ligand 11 (eotaxin), as a marker of aging in mice that appeared to be strongly related to cognitive function decline. The substance was isolated in an experiment in which the circulations of old and young mice were connected (75).

Lipids and lipid metabolism. Lipid profiles in blood have been studied extensively as risk factors for cardiovascular disease. The most important parameters that are measured in clinical practice are the triglyceride-carrying chylomicrons and very-low-density lipoproteins, the cholesterol-transporting low-density lipoproteins and high-density lipoproteins, and the free fatty acids. In several epidemiologic studies, the

relation of changes in lipid plasma levels with age has been studied, both in cross-sectional and longitudinal studies. The evidence on these as well as on other biomarkers recently was summarized by Glei et al. (76). As biomarkers of aging, these lipid profiles are of limited value. For example, the level of total cholesterol increases until the age of 50 years in men and 70 years in women (77, 78). With high-density lipoprotein cholesterol levels, no clear pattern has emerged (76). Only for triglycerides has a monotonous increase with age been found (76). Moreover, lipid levels are influenced by lifestyle and medication and strongly by genetics (43). Of potential significance is the role of free fatty acids (nonesterified fatty acids) in blood. These have been described as "metabolic villains" with reference to their presumed toxic effects (79). Free fatty acids have been studied in relation to diabetes and insulin resistance and as risk factors for several other chronic diseases (80, 81). However, their relation with aging has barely been studied.

Of note is a recent initiative through which more than 600 distinct molecular lipid species were identified in human plasma, divided over the 6 main lipid categories: sterol lipids, fatty acyls, glycerolipids, prenol lipids, glycerophospholipids, and sphingolipids (82). It seems likely that some among these, or their ratios, will be related to aging. However, at present, the laboratory techniques to determine these various molecules are far from routine.

Wear and tear: oxygen metabolism and inflammation. The oxygen radical, or oxidative stress, theory of aging goes back to an article by Denham Harman from 1956 (83). In recent years, however, some experimental results seem to refute this theory (84). Its basic tenets are that the wear and tear of metabolism produces ROS that damage DNA, membranes, and enzymes, ultimately leading to detrimental dysfunction. Whatever the status of the theory, markers for cumulative oxidative stress certainly can be useful as markers of aging. Still, measuring ROS is not easy. Free radicals are produced mainly in mitochondria, the "energy factories" of the cell, and the havoc they wreak occurs mainly within the cell.

A view that considers the overall balance between oxidants and reducers (or antioxidants) might be more relevant, inasmuch as a predominance of one over the other is a precondition for the formation of ROS. Some consensus exists on how to measure a person's antioxidant status (85). This entails assessing the major molecules involved in redox reactions, particularly glutathione (reduced and oxidized gluthation) in plasma or lymphocytes (86). In addition, components of the physiologic antioxidant system, such as uric acid, creatinine, bilirubin, albumin, and several vitamins (A, C, D, and E), can be determined in serum/plasma or erythrocytes, as can antioxidant enzymes such as glutathione peroxidase and superoxide dismutase. In lymphocytes, the antioxidant thioredoxin reductase-1 can be assessed. In clinical settings, many studies have investigated the relation between a state of oxidative stress and particular diseases, such as diabetes, atherosclerosis, and chronic inflammatory diseases (87).

One condition that is believed to lead to oxidative stress is chronic inflammation. Inflammatory processes are a component of almost all diseases associated with aging, including atherosclerosis, Alzheimer's disease, and cancer. Still, an unanswered question is whether an inflammatory state is an integral part of aging or whether it results from age-related diseases, such as atherosclerosis (88). Several markers of inflammation, well studied in large cohorts, together with relevant references are provided in Table 1.

Both oxidative stress and inflammation are associated with hypoxia. Hypoxia is an important mechanism in the pathology of cancer, heart disease, inflammatory disorders, and lung disease. It also likely has an important role in aging. Recently, much attention has been focused on the crucial role of hypoxia-inducible factor in upregulating angiogenic factors in response to ischemia (89, 90). As a transcription factor, it cannot be detected directly in plasma. However, it has been measured in leukocytes (91).

As mentioned previously, lipids also are modified by oxidative stress. Thus, isoprostanes result from the free radical-based oxidation of arachidonic acid (92). The F2-isoprostanes, which are chemically stable isomers of prostaglandin F2 and can be measured in plasma and in lymphocytes, might be especially interesting biochemical markers (35). Associations of these compounds with a wide variety of age-related diseases have been found. Moreover, several decomposition products are generated by lipid peroxidation. A few of them have been studied in humans in relation to age and disease (see Table 1).

Organ-specific biomarkers

An obstacle to extrapolating results from basic studies of aging to humans, apart from the restrictions in experimental setups and methods, is the far greater complexity of the organization of the mammalian body (37). One aspect of this greater complexity is increased specialization: Vital functions are performed by specialized organs such as the heart, the lungs, the kidney, and the brain. Failure of one of these organs is typically the cause of death. In clinical medicine, methods to assess the status of particular organs often include biochemical tests that measure the presence or concentration of markers of disease in blood or urine. In this section, we draw from the rich clinical literature on biomarkers that have been identified as useful in the diagnosis and understanding of the various age-related diseases. Our criteria for selection of relevant markers have been described in the Methods.

Oxygen transport: red blood cells. About 25 trillion red blood cells circulate in the blood at any moment (i.e., one third of the total number of cells in the body) (42). They take up, transport, and release oxygen, which binds to the hemoglobin contained in the red cells. Because red blood cells have a lifespan of 120 days on average, there is continuous turnover, with new cells that have differentiated from stem cells in the bone marrow entering the circulation. Their genesis requires erythropoietin, produced by the kidney, vitamin B_{12}, and folic acid. It has been known for a long time that the red blood cell-producing bone marrow areas shrink with age. The hematocrit (i.e., the percentage of the blood volume occupied by red cells), normally between 40% and 45%, starts to decline at age 70 years (49, 78). The concentration of hemoglobin also declines

Table 2. Biomarkers From Clinical and Epidemiologic Studies That Could Be Useful as Biomarkers of Aging

Organ System	Possible Biomarker (Material)	Level of Validation[a]	Epidemiologic Studies	Sample Type	Analytic Method	Throughput (High/Low)[b]	Further References
Oxygen transport	Hematocrit, mean cell volume, percentage of reticulocytes	1	(49, 78)	Fresh heparin blood	Hematology analyzer	Medium	(93, 96)
	Hemoglobin			Blood/erythrocytes	Autoanalyzer	High	
	Erythropoietin		(97)				
	Iron metabolism: ferritine; transferring; serum iron	3		Serum	Autoanalyzer	High	(98)
	Hepcidin			Serum	Enzyme immunoassay	High	
Blood clotting	D-dimers, plasmin-antiplasmin (measure of activated coagulation)	3	(194)	Serum/plasma	Autoanalyzer	High	
	Thrombocyte functions (enhanced platelet activity has been reported in the elderly)	3		Blood platelets	Various		(195)
	Fibrinogen, Factors VII, VIII, IX, XI, XIII, von Willebrand factor	3	(196, 197)	Plasma	Autoanalyzer	High	(198)
	Anticoagulant proteins: antithrombin III, protein S, protein C	4	Not studied in relation to aging	Plasma	Autoanalyzer	High	
	Thrombin generation test		Only studied in small numbers of volunteers	Fresh citrate plasma	Fluorometric assay (199)	Low to medium	(201)
				Fresh whole blood	Enzyme immunoassay (200)		
Immune system	Cell counts: T cells and T cell subclasses: CD3+, CD4+, CD8+, and their proportions; B cells (CD19+); monocytes (CD14+, CD16+)			Fresh anticoagulated blood	Flow cytometric assay	Low	(202)
	Naïve T cell status (defined as $CD28^{+}CD95^{-}$ cells); the proportion of these containing T-cell receptor excision circles	2	(105)	Fresh anticoagulated blood	Flow cytometric assay	Low	
	Response against specific pathogens, such as cytomegalovirus antibodies or cellular immunity against influenza virus	2	(174) (review)				(107)
	Dendritic cells			Fresh anticoagulated blood	Various functional cell culture assays using flow cytometric, enzyme immunoassay, and/or gene expression readouts	Low	(203)
	Complement system			Serum/plasma	Autoanalyzer	High	
	Immunoglobulin levels			Serum/plasma	Enzyme immunoassay	High	
	Autoantibodies			Serum/plasma	Enzyme immunoassay	High	
	Phagocytosis						

Table continues

Table 2. Continued

Organ System	Possible Biomarker (Material)	Level of Validation[a]	Epidemiologic Studies	Sample Type	Analytic Method	Throughput (High/Low)[b]	Further References
Cardiovascular system	Collagen turnover: carboxy-terminal peptide of procollagen type I; carboxy-terminal telopeptide of collagen type I; and amino-terminal peptide of procollagen type III	2	(119)	Serum/plasma	Enzyme immunoassay	High	
	Natriuretic peptides	2	(120, 122–124)	Serum/plasma	Autoanalyzer	High	(121, 204)
	Troponin	2	(125–127)	Serum/plasma	Autoanalyzer	High	
	Endothelin	4		Serum/plasma	Enzyme immunoassay	High	(113)
	Elastin in blood	4		Serum/plasma	Enzyme immunoassay	High	(115)
Lung	Surfactant D	4	(134)	Serum/plasma	Enzyme immunoassay	High	
	Apelin	3		Serum/plasma	Enzyme immunoassay	High	(137)
	Partial pressure of oxygen in arterial blood	3		Fresh arterial non-air-exposed blood (heparin anticoagulated)	Blood gas analyzer	High	(132)
Kidney	Glomerular filtration rate, creatinine, urea	1	(76, 174) (reviews)	Serum/plasma	Autoanalyzer and calculator (glomerular filtration rate)	High	(139–142)
	Neutrophil gelatinase-associated lipocalin	4		Serum/plasma	Enzyme immunoassay	High	(145)
	Cystatin C		(174) (review)	Serum/plasma	Autoanalyzer	High	
	Fibroblast growth factor 23			Serum/plasma	Enzyme immunoassay	High	(146)
	1,25-Dihydroxy cholecalciferol (vitamin D), parathormone			Serum/plasma	Autoanalyzer/enzyme immunoassay	High	
	Phosphate, phosphorus		(147, 148)	Serum/plasma	Autoanalyzer	High	
Bone and joints	Collagen: N- and C-telopeptide cross-links of type 1 collagen; procollagen type 1 N-terminal propeptide; C-terminal propeptide	2		Serum/plasma	Radioimmune assay	High	(205–207)
	Bone matrix turnover: bone-specific alkaline phosphatase; osteoclast-derived tartrate-resistant acid phosphatase form 5b; osteocalcin			Serum/plasma	Autoanalyzer/enzyme immunoassay	High	(208–211)
	Cartilage: cartilage oligomeric matrix protein	3	(154)	Serum	Enzyme immunoassay	High	
Liver	"Liver enzymes": alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transferase	3		Serum/plasma	Autoanalyzer	High	(158)
	Albumin	2		Serum/plasma	Autoanalyzer	High	(158)

Table continues

Table 2. Continued

Organ System	Possible Biomarker (Material)	Level of Validation[a]	Epidemiologic Studies	Sample Type	Analytic Method	Throughput (High/Low)[b]	Further References
Muscle	Irisin	Recently discovered marker in mice, of much potential relevance		Serum/plasma	Western blot	Low	(160)
Brain and nervous system	β-Amyloid	1	(174) (review)	Serum/plasma	Enzyme immunoassay	High	(162, 163)

Abbreviation: CD, cluster of differentiation.
[a] The degree to which the mentioned biomarkers may be more or less considered validated with regard to aging is indicated as follows: 1, widely studied and established relation with aging; 2, widely studied, but relation with aging less clear; 3, studied little, but promising as a marker of aging; and 4, has been measured in humans, but speculative as a marker of aging.
[b] Throughput: low: <50 samples/day; medium: 60–80 samples/day; high: >100 samples/day.

with age, leading to an increasing prevalence of anemia (30, 93–95). To some extent, this is due to suboptimal nutrition or chronic inflammation, but a significant part is unexplained and seems to be related to aging itself (96). In contrast to hemoglobin levels, erythropoietin levels seem to rise with age (97). To distinguish between disease and aging, markers of anemia should be studied in relation to levels of erythropoietin and of markers of iron metabolism (see Table 2). Ferritin, an iron-binding protein, has received much attention in relation to aging. It is assessed routinely in the diagnosis of anemia because low levels are a criterion for iron deficiency anemia. However, it also can become elevated in response to oxidative stress. Its concentration increases with age in men; in women, ferritin increases substantially after the menopause, with a concomitant increase in the risk for several diseases (98).

Blood coagulation. The risk of thromboembolism, not only arterial (e.g., myocardial infarction, stroke) but also venous (thrombosis and pulmonary embolism), rises with age (99). This reflects a shift in the balance between procoagulant and anticoagulant factors in the blood in favor of the former. It is therefore of much interest to study the hemostatic system as a function of age. In practice, only a few tests to assess the hemostatic status are used routinely. These are the platelet count and 2 tests with which the enzymatic clotting cascade is simulated in vitro: the prothrombin time and the activated partial thromboplastin time. However, a large array of tests is available for more detailed studies of coagulation, used mainly in the diagnosis of the numerous known (often hereditary) disorders of coagulation, including tests both of platelet function and of enzymatic clotting (100, 101). Such tests have barely been used in relation to aging. A few studies have evaluated particular aspects of the hemostatic system with regard to age, and these are mentioned in Table 2.

Immune system. The blunting of immune responses with old age has been labeled immunosenescence (102). Indications of this blunting include an inadequate response to vaccination in the very elderly. One of the first observed age-related changes of the immune system is the involution of the thymus (103, 104). Thymic involution is accompanied by a gradual loss of naïve T cells. Maintenance of an adequate pool of naïve T cells has been identified as an important characteristic of those who reach old age. This was confirmed in a recent study of nonagenarians who participated in the Louisiana Health Aging Study (105). On the other hand, an immune system that is overactive in the sense of maintaining chronic inflammation is often singled out as a hallmark of aging. Also, the prevalence of some autoimmune diseases seems to increase with age (106).

Most assessments of the immune system used in human studies depend on phenotyping and functional testing of cells of the immune system. For assessment of cellular immunity, white cells can be harvested from blood. In particular, functional studies of T and B lymphocytes have revealed several age-related differences (107). Table 2 shows several tests used to study aging of the immune system.

In blood, concentrations of the immunoglobulins of the various classes and subclasses can be measured, as well as numerous cytokines. Currently, no general consensus exists

on which aspects of the immune response provide the most significant association with age. Again, longitudinal studies are essential in determining which characteristics enhance longevity and which phenomena are (inadequate) responses to infection, stress, or other threats.

Cardiovascular system. The most noteworthy age-related changes of the vascular system are a gradual increase in the number of atherosclerotic lesions, an increase in the "stiffness" of vessels, and what has been labeled "endothelial dysfunction" (108). The first atherosclerotic lesions already occur in utero, and their number increases steadily with age. Unfortunately, studying the atherosclerotic process in vivo in humans is difficult and requires sophisticated imaging methods (109). This also applies to endothelial function. Nevertheless, endothelial progenitor cells in blood can be studied by cell culture (110–112), and the concentration of molecules regulating endothelial function, such as endothelin and angiotensin, can be measured (113). Molecules that "protect" the vessel wall from calcification, such as matrix Gla protein and fetuin-A, are potential interesting markers of aging (114).

The gradual rise of systolic blood pressure and pulse pressure with age does not produce any traces in the sense of known biochemical markers. Still, minute quantities of elastin, which is a major determinant of the vessel wall's elastic properties, appear in the blood (115). This could be a useful indicator of the aging of the blood vessel wall.

Age-related changes in the form and function of the heart are universally observed, and in about 1 in 3–5 individuals these changes ultimately lead to heart failure. Attempts to diagnose and recognize heart disease early have resulted in the discovery of several strong markers, some of which also are found in trace quantities in healthy individuals in the general population. This raises the question of whether these markers could represent an age-dependent process.

One of the age-related changes is cardiac fibrosis, which is due largely to the accumulation of collagen in the extracellular matrix, leading to increased ventricular stiffness (116). Markers of collagen turnover can be found in the blood (117–119) (Table 2). In the diagnosis and treatment of heart failure especially, the measurement of natriuretic peptides has become a fixed element of medical practice. Brain natriuretic peptide and N-terminal pro-brain natriuretic peptide are released from the wall of the heart ventricles when they experience increased pressure or are otherwise under stress. These peptides are also present in small quantities in the blood of healthy individuals (120–124).

More surprisingly, troponin, which currently is considered the gold standard for the diagnosis of myocardial infarction and which leaks into the circulation from dying cells, is traceable in the blood of a considerable percentage of people without overt cardiac disease (125–127). It can be explained by the remarkable recent discovery that, in contrast to what had been believed for the past 100 years or so, substantial turnover of cardiomyocytes occurs throughout life (128–130).

Lung function. As measured with basic spirometry, lung function consistently has been found to decline with age (131). The partial pressure of oxygen in arterial blood also declines with age, by an estimated 0.29 kPa per decade (132). Nevertheless, even though age-dependent decline is clear, it is not as dramatic as some cross-sectional studies have suggested, inasmuch as these studies are subject to major confounding (131). The effects of age impact various aspects of lung physiology, most of which require sophisticated techniques to be made visible and are beyond the scope of this review. Chronic obstructive pulmonary disease, the most prevalent age-related lung disease, has been described as an example of "accelerated aging" (133). This refers in particular to the inflammatory processes seen in chronic obstructive pulmonary disease. It is noteworthy that at least one marker has been found to be associated with pulmonary inflammation, namely surfactant protein D in serum (134). It is synthesized by type 2 alveolar cells, so it also is found in bronchoalveolar lavage fluid (135). Although its function is not precisely known, it has been shown to have antibacterial inflammation-modulating effects. It binds to dying and dead cells and inhibits the formation of lipid radicals.

In recent years, the pulmonary circulation has attracted much attention, particularly the problem of pulmonary hypertension. In a well-known population study (Olmsted, Minnesota), pulmonary artery pressure was found to increase with age, and the rate of increase was associated with death (136). A few biochemical markers of pulmonary hypertension have been suggested, among which apelin seems to be the most promising (137). However, apelin is not specific for the pulmonary circulation.

The kidney. The glomerular filtration rate is currently accepted as the most relevant measure of kidney function. The normal value in healthy young men is 130–140 mL/min per 1.73 m^2. It is estimated to decline by approximately 1 unit per year after the age of 40 years. The "functional units" of the kidney are the nephrons, of which there are more than 1 million. Decline of glomerular filtration rate with age is believed to be due largely to a decrease in the number of functioning nephrons. In a recent study of donor kidneys, it was found that the prevalence of glomerulosclerosis (replacement of glomerular cells by fibrous tissue) increased from 2.7% in the very young to 73% in the very old (138).

The accurate measurement of glomerular filtration rate is complicated. In practice, glomerular filtration rate is estimated from blood creatinine levels by one of a few alternative formulas, such as the Modification of Diet in Renal Disease equation, the Cockcroft-Gault equation, and the Chronic Kidney Disease Epidemiology Collaboration formula (139–142). Unfortunately, in many ways these formulas are unsatisfactory (143, 144).

Various senile changes in kidney structure and function were reviewed recently by Musso et al. (143). They tried to distinguish between aspects of function that do and those that do not decline with age, but the increasing prevalence of chronic kidney disease with age makes it difficult to distinguish pathologic changes from true age-dependent changes. Thus, very different conclusions have been drawn from longitudinal studies and from those that investigated kidney function in the very old, with some emphasizing the decline of several kidney functions, and others, in contrast,

finding that decline in the "healthy" is very mild (143). It might be expected that preserved kidney function is a crucial condition for reaching old age. Still, much clearly remains to be investigated, preferably in carefully designed longitudinal studies.

Several new markers of renal function are under investigation for their use as markers of disease (121). Renal injury markers that are being studied include neutrophil gelatinase-associated lipocalin, a 25-kDa protein that is normally covalently linked to gelatinase from neutrophils. It is found in serum but at very low levels in the healthy. Its expression is increased in injured epithelia and in the serum of patients with acute bacterial infections, but it is also detected in blood and urine soon after acute kidney injury (145). Another interesting molecule is cystatin C, a cysteine protease inhibitor that is produced by all nucleated cells and released into the blood at a relatively constant rate. After filtering by the glomerulus, it is almost completely reabsorbed. In contrast to creatinine, its blood levels are not affected by muscle mass.

Much excitement recently surrounded the discovery of the *klotho* gene, a supposedly longevity-conferring gene named after the Greek goddess who was one of the 3 Moirae, believed to spin the thread of life. The gene product is expressed primarily in the kidney (146). That product is a transmembrane protein that, complexed with fibroblast growth factor receptor, forms a high-affinity binding site for fibroblast growth factor 23. As such, it functions as a receptor for a hormone that regulates excretion of phosphate and is involved in the synthesis of active vitamin D. The extracellular domain of the receptor can be shed and then becomes traceable in blood and urine. This discovery has revealed a link with fibroblast growth factor 23 secretion by bone. Fibroblast growth factor 23 acts in the kidney to stimulate phosphate excretion and calcitriol (vitamin D3) synthesis. The latter increases absorption of calcium and phosphate in the intestine. The levels of phosphate in blood are further regulated by parathormone, which, in turn, is influenced by fibroblast growth factor 23/*klotho*. The main reason for the great interest this gene has evoked in aging research is that both mice deficient in fibroblast growth factor 23 and mice deficient in *klotho* develop phenotypes resembling early aging; in mice overexpressing *klotho,* aging is delayed. It was later found that the aging phenotype was largely due to hyperphosphatemia, which led to the coining of the term "phosphatopathies."

The relevance of phosphate levels in blood for disease risk in the general population also has been recognized. Thus, higher levels of phosphate, even within the normal range, have been found to be associated with increased risk of cardiovascular events (147, 148).

Bone and joint. Brittle bones are a sign of old age, entailing an increased risk of osteoporotic fractures (149). Bone tissue is always in a dynamic state, undergoing a continuous process of bone formation by osteoblasts and bone breakdown by osteoclasts. With aging, a net loss of bone mass occurs, especially of trabecular bone. Bone structure becomes disordered, and mineral density decreases. Overall, bone strength is lost. In addition to the measurements of bone mineral density by advanced imaging techniques, several biochemical markers of bone metabolism have been identified that are believed to reflect bone turnover and can be measured in blood. Some of these are markers of the activity of osteoclasts, particularly the resorption of the protein that forms the bone matrix, type 1 collagen (see Table 2). Other markers are associated with osteoblast activity, or bone formation. Levels of several of these markers change with age. Currently, the main use of these markers is in studying the effect of interventions to prevent or treat osteoporosis. It would be of much additional value to study these markers in longitudinal studies together with regulators of bone turnover, such as osteoprogerin, receptor activator of nuclear factor kappa-B ligand, and sclerostin. Besides these cellular regulators, calcium homeostasis also should be taken into consideration, in particular parathormone, serum phosphorus and calcium levels, and vitamin D metabolites ($1,25(OH)_2D$, the active form of vitamin D, and 25-hydroxyvitamin D). Finally, it is now known that osteoblasts and osteoclasts produce fibroblast growth factor 23, as mentioned above.

Besides osteoporosis, osteoarthritis is among the most prevalent age-related diseases (150). It results from degenerative processes mainly in the cartilage covering the joints and the subchondral bone (151, 152). Again, the underlying pathology is believed to be an imbalance between tissue breakdown and formation. This involves primarily the chondrocytes, which produce not only constituents of the extracellular matrix but also the enzymes to degrade them. The loss of extracellular matrix components that occurs in osteoarthritis gives rise to the appearance of markers in the serum (153). An example is cartilage oligomeric matrix protein (154). However, suitable biomarkers are still lacking. Therefore, working groups have been established to develop biomarker assays for the early diagnosis of osteoarthritis (155, 156) (http://www.nih.gov/niams; http://www.oarsi.org). It is hoped that a proteonomics-based approach could identify valid markers.

Liver. Given its renowned regenerative capacity, the liver is the organ that, at first thought, should seem to be least affected by aging. Indeed, animal studies have confirmed that the regenerative response in reaction to a toxic challenge (such as an intraperitoneal carbon tetrachloride injection) remains preserved until old age, although it becomes slower and weaker (157). The liver is further unique in that it has a double blood supply. About a quarter of its blood is supplied by the hepatic arteries; the remaining contribution is from the portal vein that directly delivers molecules that have been absorbed from the intestine for processing in the liver.

Study of liver function over time is difficult because of a lack of suitable tests. In clinical medicine, routine assessments of liver damage rest mainly on measurement of liver enzymes in the plasma, particularly alanine aminotransferase, aspartate aminotransferase, and gamma-glutamyl transferase. However, the levels of these enzymes fluctuate significantly during the day and are strongly affected by environmental factors such as diet and alcohol intake. Recently, several specialists have made a case for reevaluating the importance of aspartate aminotransferase and alanine aminotransferase as markers of overall health and risk of death (158). Because most knowledge of their relation with age

has been derived from cross-sectional determination of enzyme levels, it seems that longitudinal studies with repeated measurement could bring new insights.

Muscle. The gradual loss of muscle mass is another well-recognized aspect of aging. On average, by the age of 80 years, most people have lost about 40% of the muscle mass they had when they were young (159). When the loss of muscle starts to be problematic, the condition is referred to as sarcopenia. Most known age-related changes have been found by studying muscle biopsies and so do not qualify as suitable biomarkers. Recently however, a hormone named irisin, a cleaved membrane protein derived from muscle, was identified in human blood. In mice, its secretion has been found to be stimulated by exercise. Its main effect seems to be an increase in energy expenditure by stimulation of uncoupling protein 1 in fat tissue (160, 161). Because peroxisome proliferator-activated receptor-γ coactivator-1-overexpressing transgenic mice, in which the new molecule originally was identified, have an increased life span, irisin seems an interesting candidate for research.

Brain and nervous system. Last but not least, the brain is touched by age. All evidence indicates that mild cognitive decline is an essential aspect of aging. A strong decline is a sign of neurogenerative disease, such as Alzheimer's and Parkinson's diseases. The pathologic features of most of the neurodegenerative diseases involve aggregates of misfolded proteins, such as the senile plaques of Alzheimer's disease, which consist of aggregates of β-amyloid. Nevertheless, it is still not known whether these plaques actually cause the decline in cognitive function or whether they represent an epiphenomenon. The study of the development of these structures requires brain imaging or postmortem pathology. However, β-amyloid is detectable in blood. In the known forms of hereditary Alzheimer's disease, levels of this substance in blood are elevated (162). Levels of plasma β-amyloid have been measured in participants in the Rotterdam study, at 2 time points an average of 6.5 years apart. During this period, levels were found to have increased (163). Apart from β-amyloid, however, biochemical markers for the aging brain are lacking.

CONCLUSION

A few years ago, the American Federation for Aging Research proposed a few criteria for a "perfect" biomarker of aging (4, 164). It must predict the rate of aging (where a person is in their life span: the biologic age), and it must monitor a basic mechanism that underlies the aging process and is not an effect of disease. As long as it has not been satisfactorily elucidated what the basic aging processes are in humans, such biomarkers will remain an illusion.

Most researchers agree that aging is caused by a lifelong accumulation of molecular and cellular damage (165). Compromised genes and cellular pathways give rise to changes in tissues, resulting in increased vulnerability to disease and ultimately dysfunction of organs. Dysfunctional organs, in turn, can affect the functioning of other bodily systems. Thus, the organ damage occurring in chronic age-related diseases could be largely the result of a gradual disruption of normal molecular processes and adaptations to the resulting damage. If no essential distinction exists between organ aging in age-related chronic diseases and general mechanisms of aging, then studying pathologic changes in organs in chronic disease also could provide valuable insights into aging in general.

In this review, we have taken a broad approach, including markers used to detect organ-specific disease. Obviously, this greatly expands the scope of accessible biomarkers and provides a potentially fruitful link with clinical medicine, facilitating the translation from the insights gained from laboratory animals to human aging and disease. One of the emerging features that arises when translating findings in lower experimental species to humans is the far greater organizational complexity of the mammalian body. Which of the organs is "the weakest link"? Barring all known causes of death, of what do we die? Is it a failing heart or lung or liver? Or is it rather a general kind of metabolic failure or system breakdown?

We have reviewed both biomarkers that have been assessed more or less extensively in epidemiologic (cohort) studies and biomarkers that have emerged from animal models as potentially important in humans. Although the former have revealed many age-related changes, no biomarker has emerged that comes close to the ideal described previously. Perhaps the most is to be expected from a panel of markers, some of which might be organ specific. As far as potential new biomarkers are concerned, developments in transcriptomics, epigenetics, proteomics, lipidomics, metabolomics, and related disciplines could greatly enhance the analysis of aging processes in laboratory animals. These new disciplines already are pointing the way to new biomarkers that can be assayed in humans.

In all cases, longitudinal studies with repeated measurements of relevant biomarkers could greatly contribute to unraveling the complex web of aging and disease processes in humans. The other way around, longitudinal studies are needed to validate proposed biomarkers.

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