Journal of Gerontology: BIOLOGICAL SCIENCES
2007, Vol. 62A, No. 10, 1096–1105

A Method for Identifying Biomarkers of Aging and Constructing an Index of Biological Age in Humans

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This study was conducted to identify biomarkers of aging and to construct an index of biological age in humans. Healthy adult men ($n = 86$) who had received an annual health examination from 1992 through 1998 were studied. From 29 physiological variables, five variables (forced expiratory volume in 1 second, systolic blood pressure, hematocrit, albumin, blood urea nitrogen) were selected as candidate biomarkers of aging. Five candidate biomarkers expressed substantial covariance along one principal component. The first principal component obtained from a principal component analysis was used to calculate biological age scores (BAS). Individual BAS showed high longitudinal stability of age-related changes. Age-related changes of BAS are characterized by three components: age, peak functional capacity, and aging rate. A logistic regression analysis suggested that aging rate was influenced by environmental factors, but peak functional capacity was almost independent of environmental factors.

A multivariate index of biological age as a generalized measure of senescence would be a highly useful tool for gerontological research (1–4). Biological age would represent a mathematical composite of biomarkers of aging for which evidence had been presented to establish the reliability and validity of each of the biomarkers. If such an index of biological age could be established, then it might be possible to determine the rate of aging within individuals or groups of individuals and also to monitor the effectiveness of purported interventions on aging processes (3,5,6). The number of studies examining possible interventions to slow the rate of aging in long-lived species has increased recently and include investigations of calorie restriction, exercise, drugs, and hormones; however, a standardized method for examining the effectiveness of these interventions has not yet been established (2–4,7).

The concept of ''biological'' age or ''functional'' age has met with considerable controversy regarding its validity and utility (7–11). This controversy stemmed in part from early attempts to define a single index of biological or functional age for humans based on multiple regression analyses derived from cross-sectional studies of specific human populations (e.g., 12–14). Costa and McCrae (15,16) pointed out that the evidence from such studies indicates that these analyses do not provide better information about biological age than does chronological age itself.

In light of the controversy regarding methods for assessing biological age, it is evident that this research area has lacked a logical strategy inclusive of statistical models for developing biomarkers of aging and providing a demonstration of their reliability and validity. In the simplest application, a biomarker of aging is often viewed as any biological parameter that is significantly correlated with adult age. In previous reports addressing this issue (2,17,18), this definition has been expanded considerably to provide more stringent statistical criteria to a candidate biomarker of aging that supports its reliability and validity. Specifically, this strategy has proposed that candidate biomarkers of aging must meet at least the following four statistical criteria: (i) significant cross-sectional correlation with age; (ii) significant longitudinal change with age consistent with the cross-sectional correlation; (iii) significant stability of individual differences in the measure; and (iv) rate of age-related change proportional to differences in life span among related species. These criteria relate to both the reliability and validity of candidate biomarkers of aging. In following this strategy, previous reports addressed potential biomarkers of aging in rhesus monkeys. In the present study, we follow this strategy to identify potential biomarkers of aging in healthy Japanese men for use in developing an index of an individual's biological age.

Methods

Participants

Among about 18,000 Japanese adult men who received a routine health check-up from 1992 through 1998 at the Kyoto Second Red Cross Hospital, 122 who received a 2-day routine health check-up once a year (April to May) successively for 7 years from 1992 through 1998 were randomly selected as potential participants. Each man's past and present health status, work history, social and dietary habits, e.g., were determined from the medical questionnaire. Written informed consent was obtained from all potential participants.

From this sample of 122 potential participants, we eliminated 36 after further examination identified them as having abnormal measurements related to such diseases as hypertension, diabetes, asthma or chronic coughing, and

Table 1. Age and Physical Characteristics of the Participants at Baseline Measurement

	Age Group				
Variable	30–39	40–49	50–59	60–69	70<
Age, y					
Mean	35.9	44.8	54.2	63.6	73.3
SD	2.3	2.9	2.7	2.8	2.8
Height, m					
Mean	1.71	1.68	1.66	1.64	1.62
SD	0.04	0.06	0.05	0.06	0.06
Body weight, kg					
Mean	66.9	65.8	65.9	61.5	60.3
SD	8.8	6.6	6.7	7.4	8.8
Participants, n	17	26	18	16	9

Note: SD = standard deviation.

excessive obesity. We also eliminated those men who did not have complete data for all the variables examined during the 7-year observation period. As a final sample, 86 men were selected as participants. These men have been described in more detail elsewhere (19). Most of them were judged as healthy based on clinical criteria for normality set by the Japanese Red Cross Hospital (19). However, several elderly men with hypertension, diabetes, and hyperlipemia tendencies were included in the participant group. In the case of elderly people, it is difficult to distinguish between normal and abnormal aging.

Most participants resided in Kyoto City. Their occupations were: managers (12.8%), salesmen (20.9%), researchers and engineers (5.8%), storekeepers (11.6%), teachers (5.8%), unemployed (18.6%), and various others (24.5%). The age range of participants in several age cohorts at the beginning of this study was from 31 to 77 years, with a mean age of 51.2 years. The number of participants by age group and their physical characteristics are given in Table 1.

Test Items and Procedure

The 2-day health examination consisted of more than 60 test items, including anthropometric measurements, cardiovascular and respiratory functions, and physical and chemical properties of blood and urine. Excluding results of tests expressed by binary variables, and considering the connection of the results of tests with the aging process, the following items tested in the routine check-up were assessed in the current study:

Results of cardiorespiratory function tests: systolic blood pressure (SBP, mmHg); diastolic blood pressure (DBP, mmHg); forced vital capacity per square of height (FVC/Ht^2, L/m^2); forced expiratory volume in 1.0 second per square of height (FEV_1/Ht^2, L/m^2).

Results of hematology assays: white blood cell count (WBC, $10^2/mm^3$); red blood cell count (RBC, $10^4/mm^3$); hemoglobin concentration (HB, g/dL); hematocrit (HCT, %); mean corpuscular volume (MCV, fl); mean corpuscular hemoglobin (MCH, pg); mean corpuscular hemoglobin concentration (MCHC, %).

Results of biochemical assays of serum: total protein (TPRO, g/dL); albumin (ALBU, g/dL); globulin (GLOB, g/dL); ratio of albumin to globulin (A/G); total bilirubin (TBILI, mg/dL); alkaline phosphatase (ALK, IU/L); γ-glutamyl transpeptidase (GTP, IU/L); glutamate oxaloacetate transaminase (GOT, IU/L); glutamic pyruvic transaminase (GPT, IU/L); lactic dehydrogenase (LDH, IU/L); blood urea nitrogen (BUN, mg/dL); creatine (CREAT, mg/dL); uric acid (URIC, mg/dL); calcium (CALC, mg/dL); total cholesterol (TC, mg/dL); triglyceride (TG, mg/dL); high density lipoprotein cholesterol (HDLC, mg/dL); and blood glucose (GLU, mg/dL).

Pulmonary function (FVC and FEV_1) was measured using an electric spirometer (System-9; Minato Co. Ltd., Osaka, Japan) three times while the participant was standing, and the best record was used. Reproducibility was judged by the criteria of the American Thoracic Society cited by Ferris (20). In this analysis, FVC and FEV_1 were divided by the square of height to remove the effects of body size. Blood pressure (SBP and DBP) was measured manually using a sphygmomanometer after a 10-minute rest in a sitting position. Standard hematology and blood chemistry assays were performed at the Medical Laboratory of the Kyoto Red Cross Hospital. Biochemical measurements of heparinized blood were carried out using a Hitachi Automatic Analyzer (Model-7150; Tokyo, Japan). The hematological measurements were made on a Symex Automatic Blood Analyzer (E-4000; Tokyo, Japan).

Furthermore, a self-administered questionnaire given to participants in 1992 and 1998 to obtain information on bias factors of biological aging such as age, disease presence, occupation, smoking status, drinking habits, and level of exercise. This information was classified as dichotomized variables (0 or 1). For example, Exercise is 0 = exercisers (men taking part in activities such as jogging, walking, or tennis > 2 times per week) or 1 = none.

Statistical Analysis

For systematic and logical selection of biomarkers of aging, the following stepwise methods were used as described in the Results section: (i) cross-sectional analysis; (ii) longitudinal analysis; (iii) stability analysis; and (iv) assessment of redundancy. For all analyses, statistical significance was accepted as $p < .05$. All the computations (inclusive of a principal component analysis (PCA) and a multiple logistic regression analysis) were made with computer programs in the Statistical Package for the Social Sciences (21).

Results

Selection of Candidate Biomarkers of Aging

Table 2 provides the set of correlations used to guide the first three steps of the selection process to identity candidate biomarkers of aging.

Step 1: Cross-sectional analysis.—To identify the degree of relationship between each variable and chronological age, we first examined cross-sectional data for each year (from 1992 through 1998). Specifically, values for the 29 physiological, hematological, and blood chemistry variables were correlated with the chronological age of each participant for

Table 2. Summary of Correlation Coefficients Obtained From Longitudinal, Cross-Sectional, and Stability Analyses for 86 Healthy Adult Men

Variable	Longitudinal Analyses		Cross-Sectional Analysis N = 86	Stability Analysis N = 86
	Individual Analysis N = 86	Cross-Longitudinal Analysis N = 602		
1 Systolic blood pressure	0.580**	0.388**	0.358**	0.807**
2 Diastolic blood pressure	0.405**	0.181*	0.152	0.797**
3 FVC/Height^2	−0.508**	−0.530**	−0.518**	0.862**
4 FEV_1/Height^2	−0.626**	−0.709**	−0.702**	0.819**
5 White blood cell count	−0.115	−0.061	−0.051	0.747**
6 Red blood cell count	−0.367**	−0.304**	−0.324**	0.869**
7 Hemoglobin	−0.229*	−0.186**	−0.221*	0.832**
8 Hematocrit	−0.435**	−0.271**	−0.290**	0.815**
9 Mean corpuscular volume	−0.139	0.187**	0.205	0.903**
10 Mean corpuscular hemoglobin	0.274*	0.171**	0.159	0.848**
11 Mean corpuscular hemoglobin con.	0.422**	0.025	−0.024	0.557**
12 Total protein	0.019	−0.148**	−0.157	0.651**
13 Albumin	−0.310**	−0.409**	−0.441**	0.817**
14 Globulin	0.112	0.113*	0.175	0.657**
15 Albumin/Globulin ratio	−0.222*	−0.219**	−0.225*	0.685**
16 Total bilirubin	−0.041	−0.029	0.017	0.641**
17 Alkaline phosphatase	−0.333**	0.141**	0.163	0.872**
18 γ-glutamyl transpeptidase	−0.206	−0.028	−0.023	0.841**
19 Glutamate oxaloacetate transaminase	0.101	0.108*	0.092	0.597**
20 Glutamic pyruvic transaminase	−0.037	−0.061	−0.061	0.697**
21 Lactic dehydrogenase	−0.245*	0.152**	0.187	0.775**
22 Blood urea nitrogen	0.251*	0.347**	0.358**	0.703**
23 Creatine	0.181	0.275**	0.269*	0.845**
24 Uric acid	0.009	−0.170**	−0.173	0.822**
25 Calcium	−0.173	−0.184**	−0.176	0.681**
26 Total cholesterol	−0.165	0.108*	0.122	0.644**
27 Trigyceride	0.003	−0.121**	−0.121	0.685**
28 High-density lipoprotein cholesterol	0.179	0.138**	0.127	0.841**
29 Blood glucose	0.129	0.179**	0.171	0.849**

Notes: *$p < .05$; **$p < .01$. FVC = forced vital capacity; FEV_1 = forced expiratory volume in 1 second.

each year across all age groups. Thus we produced seven correlations for each variable. These seven correlations (Pearson product-moment) obtained for each of the 7 years were then averaged to obtain an estimate of the mean cross-sectional correlation with chronological age by using Fisher's transformation of r to z and z to r. Based on this criterion, we identified the following 10 variables for further analysis: SBP, FVC/Ht^2, FEV_1/Ht^2, RBC, HB, HCT, ALBU, A/G ratio, BUN, and CREAT ($p < .05$). The highest correlation with chronological age was observed for FEV_1/Ht^2 (–0.70). The next highest correlations observed were for FVC/Ht^2 (–0.52) and ALBU (–0.44).

Step 2: Longitudinal analysis.—To identify the degree of genuine age-related ''change'' in each variable, we conducted a longitudinal analysis for each variable across 7 years, the results of which are shown in Table 2. We used two methods for this analysis. With the first method, we first transformed the measurement variable and chronological age of each participant across 7 years into z scores to standardize the scales. We then calculated correlations between chronological age and the values for each participant. Using Fisher's transformation of r to z and z to r, we then calculated means of 86 individual r values (individual analysis). With the second method (cross-longitudinal analysis), we analyzed correlations between chronological age and the measurement value for each variable across 7 years (using the 7-year longitudinal data of 86 participants). This analysis was labeled as a cross-longitudinal analysis because it involved a combination of cross-sectional and longitudinal data. The criterion for selection of candidate biomarkers at this step was a statistically significant correlation in both the individual and cross-longitudinal analyses. From the individual analysis, we identified 14 variables for further analysis: SBP, DBP, FVC/Ht^2, FEV_1/Ht^2, RBC, HB, HCT, MCH, MCHC, ALBU, A/G ratio, ALK, LDH, and BUN. The highest correlation with chronological age was observed for FEV_1/Ht^2 (–0.63), followed by SBP (0.58). Other relatively high correlations with chronological age were observed for FVC/Ht^2 (–0.51), HCT (–0.44), and DBP (0.41). From the cross-longitudinal analysis, we identified 24 variables. However, the following 9 variables; SBP, FVC/Ht^2, FEV_1/Ht^2, RBC, HCT, ALBU, A/G ratio, BUN, and CREAT, which showed a correlation of 0.2 ($p < .01$), were selected at that point. Two variables, ALK and LDH, showed different slopes between the individual analysis and cross-longitudinal analysis, although these variables showed significant correlations with chronological age. The highest correlation with chronological age was observed for FEV_1/Ht^2 (–0.71) followed by FVC/Ht^2 (–0.53), ALBU (–0.41), and SBP (0.39). These correlations were similar to those observed in the cross-sectional analysis at Step 1.

Comparing the results of Steps 1 and 2, nine variables met the criteria for significant longitudinal change with age

Table 3. Means, Standard Deviations and Correlation Matrices of Nine Candidate Biomarkers of Aging and Chronological Age Calculated From the 7-Year Longitudinal Data of 86 Healthy Adult Men

Biomarker	Means	SD	1	2	3	4	5	6	7	8	9
1 Age, y	54.12	12.07									
2 Systolic blood pressure, mmHg	126.85	14.83	0.388								
3 FVC/height^2, L/m^2	1.35	0.21	−0.531	−0.298							
4 FEV_1/height^2, L/m^2	1.06	0.19	−0.709	−0.393	0.849						
5 Red blood cell count, 10^4/mm^3	478.61	32.82	−0.304	−0.018	0.226	0.289					
6 Hemoglobin, g/dl	15.05	0.97	−0.186	−0.049	0.112	0.149	0.711				
7 Hematocrit, %	44.49	2.71	−0.272	−0.141	0.137	0.191	0.701	0.862			
8 Albumin, g/dl	3.91	0.23	−0.409	−0.108	0.338	0.415	0.271	0.234	0.218		
9 Albumin/globulin ratio	1.19	0.17	−0.219	−0.196	0.217	0.262	0.031	0.013	−0.025	0.619	
10 Urea nitrogen, mg/dl	15.92	3.35	0.347	0.086	−0.133	−0.187	−0.077	−0.104	−0.188	−0.183	−0.069

Notes: R = 0.217 required for $p < .05$. SD = standard deviation; FVC = forced vital capacity; FEV_1 = forced expiratory volume in 1 second.

consistent with cross-sectional correlation: SBP, FVC/Ht^2, FEV_1/Ht^2, RBC, HB, HCT, ALBU, A/G ratio, and BUN.

Step 3: Stability analysis.—Next we examined the degree of longitudinal stability of individual differences in all the variables. For this analysis, we evaluated the inter-year reliability of the annual values for each variable. Specifically, correlations were calculated between the measurement value obtained for each of the variables and the corresponding measurement value of the succeeding year, for example, between 1992 and 1993, 1993 and 1994, across all ages within participants. Then we applied a partial correlational analysis using chronological age as a covariate to control the effects of age. To calculate a mean value of the partial correlation coefficients across the 7 years, we applied the Fisher transformation of r to z and z to r.

Stability of measurements was observed for all the variables evaluated, ranging from 0.557 for MCHC to 0.903 for MCV. These results indicate a high degree of reliability for all the measurements made. Thus, we found that all nine variables emerging from Steps 1 and 2 showed statistically significant stability ($p < .01$) with a correlation coefficient of > 0.7.

Therefore, based on the statistical criteria of a significant cross-sectional correlation with chronological age, a significant longitudinal change with chronological age, and significant stability of individual differences, nine variables–SBP, FVC/Ht^2, FEV_1/Ht^2, RBC, HB, HCT, ALBU, A/G ratio, and BUN–were further assessed as candidate biomarkers of aging.

Step 4: Assessment of redundancy.—For the set of variables identified in the preceding step, a correlation matrix was generated for all participants to examine their interrelationships and to identify possible redundant variables (Table 3). A priori we had planned to eliminate from further analysis those variables that appeared to be redundant, i.e., from the same system (FVC/Ht^2 and FEV_1/Ht^2; RBC, HB, and HCT; and ALBU and A/G ratio). These variables showed a high correlation with each other (i.e., the correlation between FVC/Ht^2 and FEV_1/Ht^2 was 0.849, the correlation between RBC and HB was 0.711, the correlation between RBC and HCT was 0.701, the correlation between HB and HCT was 0.862, and the correlation between ALBU and A/G ratio was 0.619). Therefore, we selected FEV_1/Ht^2 instead of FVC/Ht^2, HCT instead of RBC and HB, and ALBU instead of A/G ratio because these three variables had much higher coefficients of correlation in the other analyses than did FVC/Ht^2, RBC, HB, and A/G ratio in the individual analysis, possibly reflecting the rate of aging. Thus, through this statistical screening process, we identified the following five candidate biomarkers of aging for use in constructing an index of biological age: SBP, FEV_1/Ht^2, HCT, ALBU, and BUN.

PCA

A PCA was applied to the five candidate biomarkers of aging identified using the above criteria. This analysis was conducted to determine the structure of the covariance. For the first analysis, chronological age was included to confirm the relationship between age and the principal component identified. For the second analysis, chronological age was excluded to ascertain whether the relationships of the candidate biomarkers to principal components would hold without the influence of chronological age.

The results of the first analysis indicated that only one principal component was identified (Table 4). Along with chronological age, all the candidate biomarkers of aging showed significant loading onto the first principal component, which explained 42.1% of the total variance. Results from the second analysis presented in Table 5 revealed that all the candidate biomarkers maintained significant factor loadings onto the first principal component even when chronological age was eliminated. Moreover, this component continued to account for a high degree of the total variance (37.6%), with no other principal component emerging from the analysis with an Eigen-value > 1.0, following Guttman's law of lower bound for the number

Table 4. Principal Component Analysis of Five Candidate Biomarkers and Chronological Age

Variable	First Principal Component
Age, y	0.871
Systolic blood pressure, mmHg	0.542
FEV_1/height^2, L/m^2	−0.824
Hematocrit, %	−0.452
Albumin, g/dl	−0.616
Urea nitrogen, mg/dl	0.462
Eigenvalue	2.527
% Total variance	42.12

Note: FEV_1 = forced expiratory volume in 1 second.

Table 5. Principal Component Analysis of Five Candidate Biomarkers

Variable	First Principal Component	F1 Score Coefficient
Systolic blood pressure, mmHg	0.561	0.299
$FEV_1/height^2$, L/m^2	−0.782	−0.416
Hematocrit, %	−0.526	−0.281
Albumin, g/dl	−0.669	−0.356
Blood urea nitrogen, mg/dl	0.477	0.254
Eigenvalue	1.879	
% Total variance	37.58	

Note: FEV_1 = forced expiratory volume in 1 second.

of factors (22). From these results, we deduced that the five variables represented an underlying factor that might reflect processes of biological aging. The data obtained for the five candidate biomarkers of aging (SBP, FEV_1/Ht^2, HCT, ALBU, and BUN) across the 7 years of the study are presented in Figure 1 as a function of chronological age. To assess the longitudinal features of each biomarker, we calculated a regression line of the biomarker onto chronological age for each individual across 7 years. These regression lines are plotted in Figure 2.

Constructing the Biological Age Score

Because the five candidate biomarkers of aging were considered to measure underlying biological aging processes that were related statistically, we proceeded to combine them into a multivariate index, designated as a biological age score (BAS). To calculate individual BAS values, each test score for an individual was first standardized and then summed across tests in a weighted manner using the coefficients of the factor scores obtained in the PCA (Table 5). In this procedure, we reduced the equation used to calculate individual BAS to a simple equation as follows:

$$\mathrm{BAS} = 0.02\mathrm{SBP} - 2.189\mathrm{FEV}_1/\mathrm{Ht}^2 - 0.104\mathrm{HCT} - 1.541\mathrm{ALBU} + 0.077\mathrm{BUN} + 9.19$$

Individual BAS values for 86 men based on 7-year longitudinal data were calculated using this equation. Figure 3 displays the scattergram of the BAS values onto chronological age for all participants. In the cross-sectional analysis based on 7 years of longitudinal data, individual BAS values were scattered relatively symmetrically above and below the regression line. The correlation coefficient between BAS values and chronological age was 0.72 ($p < .01$), and the standard error of the estimate (*SEE*) was 0.69.

Age-Related Change in the BAS and Its Longitudinal Stability

This BAS equation might be useful for assessing the state of biological aging of an individual; however, it is difficult to directly estimate the longitudinal changes of the BAS from the scattergram presented in Figure 3. Thus, to assess the longitudinal features of the BAS in greater detail, we calculated the regression line of the BAS onto chronological

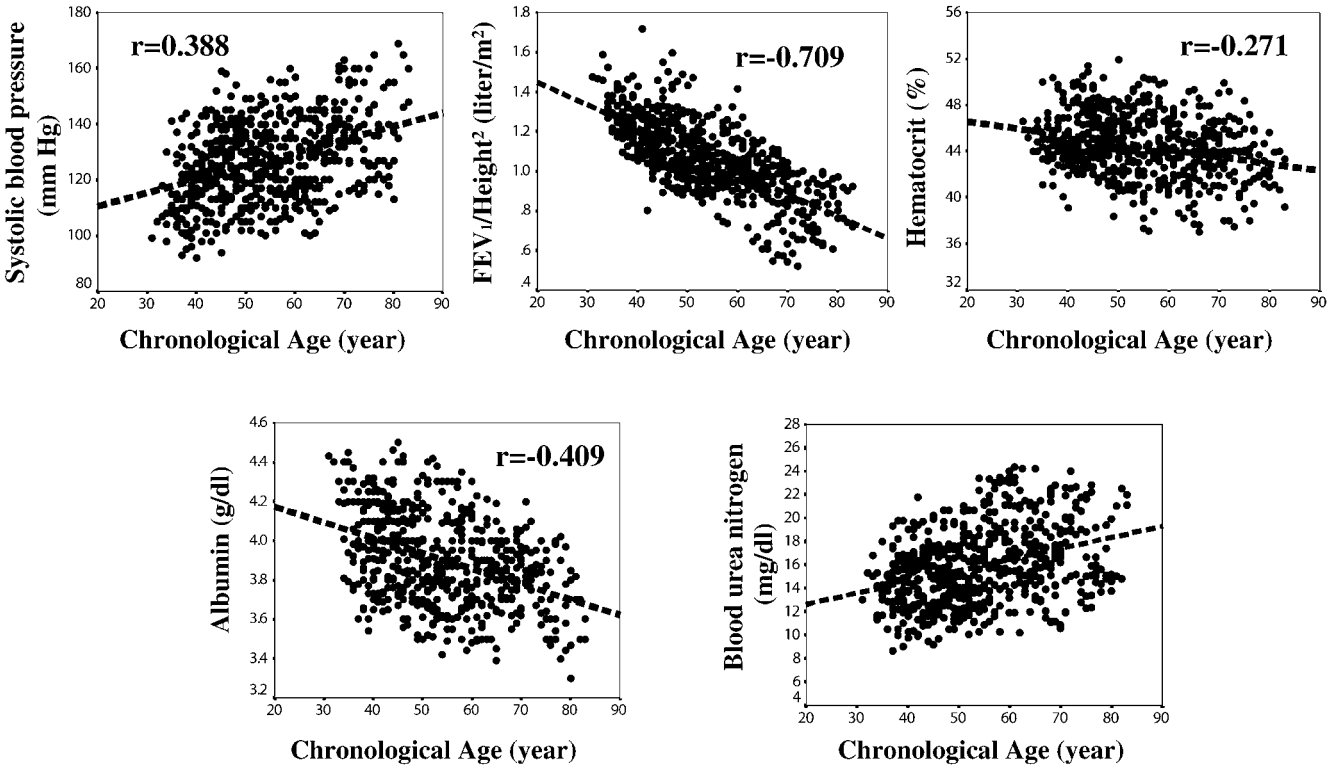

Figure 1. Scatterplots and regression lines of the five selected candidate biomarkers of aging—systolic blood pressure (SBP), forced expiratory volume in 1.0 second per square of height (FEV_1/Ht^2), hematocrit (HCT), albumin (ALBU), and blood urea nitrogen (BUN)—based on a cross-sectional data analysis. Results were obtained from 7-year longitudinal data of 86 participants.

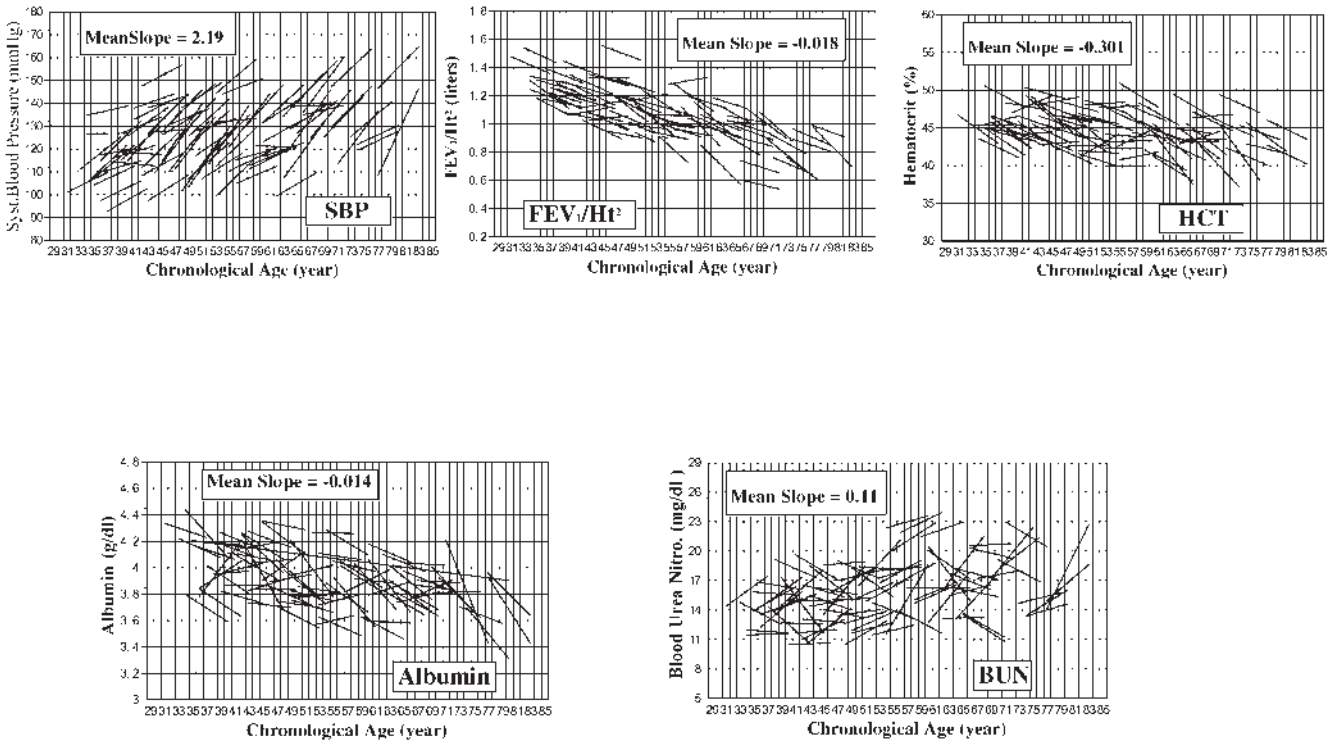

Figure 2. Scatterplots of the five selected candidate biomarkers of aging—systolic blood pressure (SBP), forced expiratory volume in 1.0 second per square of height (FEV_1/Ht^2), hematocrit (HCT), albumin (ALBU), and blood urea nitrogen (BUN)—based on longitudinal data analyses. Only the regression lines for biomarkers onto age for each individual across 7 years were plotted.

age for each individual across 7 years. These regression lines are plotted in Figure 4. All participants showed positive slopes, indicating that BAS values increased with chronological age; however, individual variability in the slopes was evident, and the pattern of variability changed with increasing chronological age.

To compare age-related changes of BAS values across age, the participants were divided into three age groups: age < 45 (young group, $n = 20$), $45 \leq$ age < 65 (middle-aged group, $n = 47$), and $65 \leq$age (old group, $n = 19$), based on the participant's age corresponding to the midpoint in the 7-year period of data collection. The mean slopes of the regression lines of BAS values estimated for each of the three age groups were 0.114, 0.145, and 0.211, respectively. An analysis of these mean slopes for the three groups by one-way analysis of variance confirmed a significant group

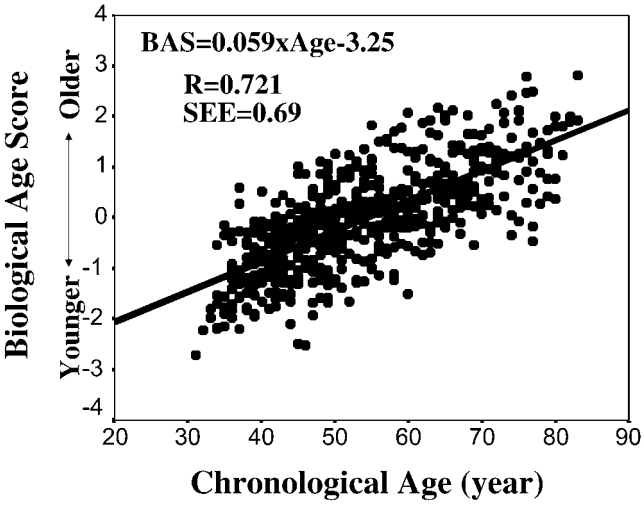

Figure 3. Relationship between biological age score (BAS) and chronological age in a cross-sectional analysis of 86 healthy men. SEE, standard error of the estimate.

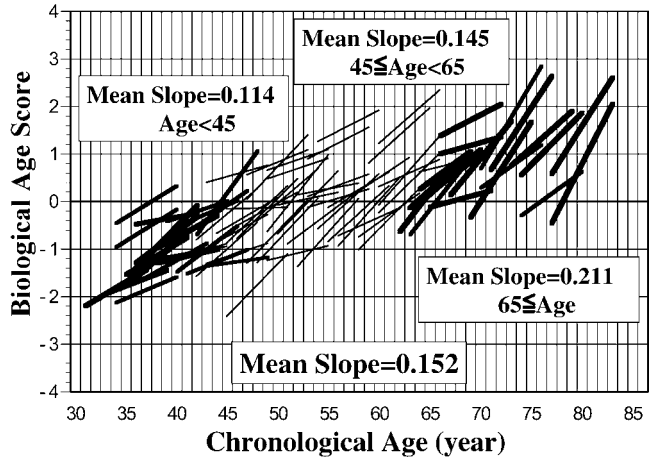

Figure 4. Relationship between biological age score (BAS) and chronological age in a longitudinal analysis of 86 healthy men. Individual BAS values were eliminated to avoid a complex configuration. Only the regression lines for BAS with age were plotted.

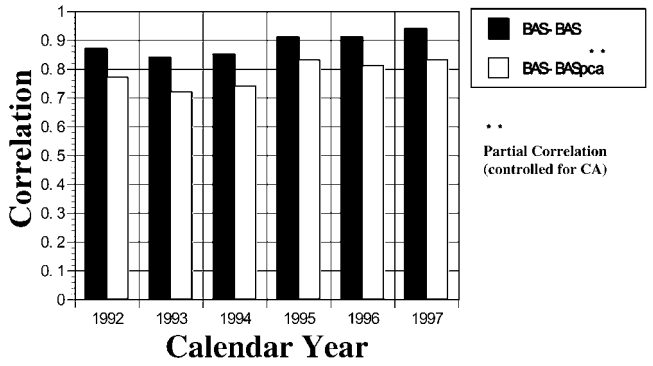

Figure 5. Assessment of the stability of the biological age score (BAS) across the 7-year period. Two sets of correlations: (i) the interyear correlations of the BAS between succeeding years, e.g., 1st year (1992) versus 2nd year (1993) of the study (BAS-BAS); and (ii) the inter-year correlations of the BAS between succeeding years with chronological age (CA) partialed out (BAS-BASpca). $R = .283$ required for $p < .01$.

effect, $F_{2, 83} = 10.622$, $p < .001$. Furthermore, analysis of the differences between all pairs of means by the Tukey's method indicated that there was a significant difference ($p < .01$) between the young and old groups, and between the middle-aged and old groups. These results indicate that the rate of aging measured by the BAS was about 1.8-fold higher in the old group compared to the young group.

We also assessed the stability of the BAS values across the 7-year period of data collection. Figure 5 presents two sets of correlations: (i) the inter-year correlations of BAS values between succeeding years, e.g., 1st year versus 2nd year of the study (BAS-BAS); and (ii) the inter-year correlations of BAS between succeeding years with chronological age partialed out (BAS-BASpca). A mean inter-year correlation of > 0.88 was observed. Even when chronological age was partialed out of the correlation, the correlations of BAS between succeeding years were very high (> 0.7). These findings indicate that the stability of the BAS within an individual was very high.

Analysis of Individual Differences in the BAS

As is evident from Figures 3 and 4, there is a considerable difference in the age-related changes of BAS values among individuals of the same chronological age. Figure 6A shows that the mean BAS of all the individuals at the same age should be equal to their chronological age. Figure 6B shows that different individuals differ in peak functional capacity. Figure 6C shows that the rate of aging varies widely among individuals. It is considered that these individual differences are caused mainly by genetic and environmental factors. However, it is difficult to measure the direct effect of heredity and the environment on the individual differences in BAS values. To solve this problem, we first hypothesized that peak functional capacity and aging rates in terms of the BAS were determined only by environmental factors. We next tried to examine the influence of environmental factors on both high aging rates and high peak functional capacities. Then, a multiple logistic regression model was used to analyze the effect of environmental factors such as disease, occupation, smoking status, drinking habits, and level of exercise on high aging rates and high peak functional capacities, using the data obtained from the self-administered questionnaire which was completed by 122 participants in 1998. Individual BAS values for 120 participants (two participants were excluded because data for them was missing) were calculated using the healthy participants equation devised in the present study, and an individual slope for each participant was calculated as a coefficient of the regression line of the BAS onto chronological age across 7 years. Also, a mean slope was calculated from the slope data for 120 participants. The participants with slopes greater than the mean slope (0.155) were considered to have high

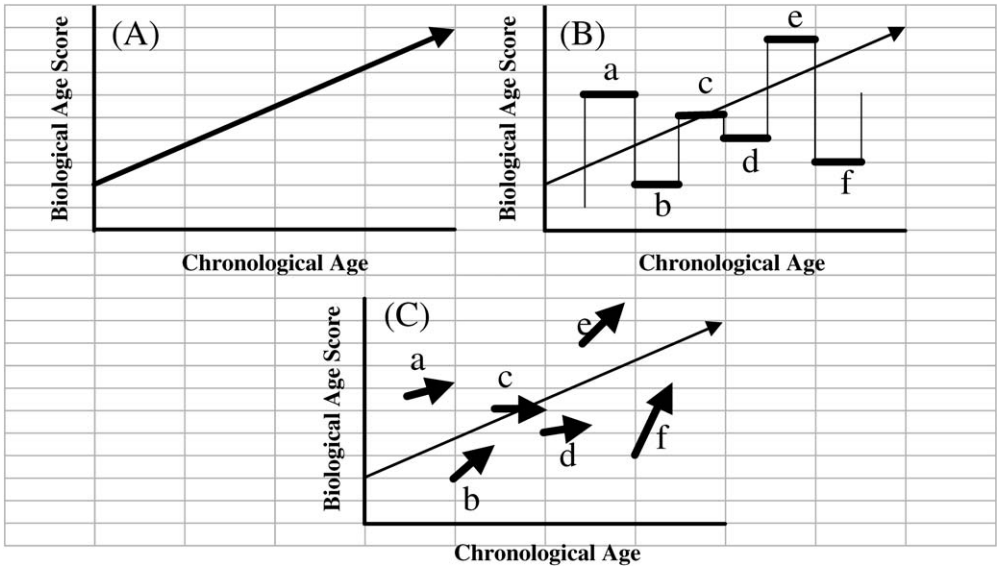

Figure 6. Hypothetical diagram showing causes of individual differences in the biological age score. An individual's biological age is determined by three components: age (A), peak functional capacity (B), and aging rate (C).

Table 6. Shows Adjusted Odds Ratio for the Presence of (a) High Aging Rate and (b) High Peak Functional Capacity in Association With Phenotypic Factors Among 120 Japanese Men

		a) Presence of High Aging Rate				b) Presence of High Peak Functional Capacity			
Covariates	No. of Participants	No. of Cases	Adjusted OR[†]	95% CI[‡] Lower Upper	*p* Value	No. of Cases	Adjusted OR[†]	95% CI[‡] Lower Upper	*p* Value
1. Age, +1 y	120	61	1.054	(1.021, 1.089)	< .001*	61	0.991	(0.964, 1.019)	.524
2. Disease									
None	105	52	1			54	1		
Yes, I have	15	9	1.321	(0.334, 5.236)	.554	7	0.794	(0.257, 2.452)	.912
3. Occupation									
Yes, I have	74	35	1			35	1		
None	46	26	1.147	(0.487, 2.702)	.767	26	1.525	(0.703, 3.308)	.295
4. Smoking									
Nonsmokers	88	42	1			47	1		
Smokers	32	19	2.749	(1.029, 7.344)	< .041*	14	0.661	(0.284, 1.532)	.386
5. Drinking									
Nondrinkers	32	19	1			16	1		
Drinkers	88	42	0.632	(0.247, 1.618)	.386	45	1.021	(0.443, 2.347)	.877
6. Exercise									
Exercisers	34	10	1			18	1		
Nonexercisers	86	51	3.052	(1.202, 7.751)	< .018*	43	0.918	(0.399, 2.116)	.855

Notes: [†]Adjusted odds ratio obtained from a multiple logistic regression model that included age (continuance variable) and dichotomized variables of disease status, occupation status, smoking status, drinking status, and exercise status as explanatory variables. Men with a slope above the mean BAS slope were considered to have a high aging rate. Men with high BAS scores over the regression line of BASs on age were considered to have a high peak functional capacity.

[‡]95% confidence interval.

*Null hypotheses of regression coefficients by Wald test were rejected at $p < .05$ level of significance.

CI = confidence interval; OR = odds ratio.

aging rates. Moreover, based on the individual BAS values for 120 participants estimated using the data for an annual health examination in 1995 (corresponding to the midpoint in the 7-year period of data collection), a regression line of their BAS values onto their chronological age was calculated. Participants with a BAS score greater than this regression line were considered to have high peak functional capacity.

Table 6 shows adjusted odds ratios (ORs) for the presence of men with high aging rates and high peak functional capacities in terms of BAS values after adjusting for all covariates. Significant predictors ($p < .05$) and their OR values for a high aging rate were age (1.054), current smoking status (2.749), and nonexercisers (3.052). For an age parameter, when age increases by 1 year, an increase in the rate of aging of 1.054-fold was dangerous. However, significant predictors for high peak functional capacity were not recognized. These results suggested that the aging rate was influenced by environmental factors, but the peak functional capacity was almost independent of environmental factors.

Discussion

Efforts to develop the best possible strategy for identifying biomarkers of aging have generated considerable disagreement about the logic involved and the value of any approach (1,7–11,15,23). In its simplest application, a biomarker of aging is defined as a biological parameter that is correlated with chronological age as evidenced in a cross-sectional analysis. However, gerontologists have long recognized the problem of relying solely on cross-sectional data to draw conclusions about aging processes, particularly in humans and other long-lived species. A correlation between a biological parameter and chronological age emerging from a cross-sectional analysis can reveal age differences in the parameter only at a specific point in time. A cross-sectional analysis cannot be used to conclude that changes have occurred because of aging phenomena. Therefore, longitudinal designs can provide a more complete explanation of age-related changes over time (16,24). In the present study, we adapted to humans a strategy previously developed for a longitudinal analysis of aging in rhesus monkeys.

A logical strategy for identifying candidate biomarkers of aging and evaluating their reliability and validity has been proposed for rhesus monkeys (2,17,18). Using a statistically defined approach, four criteria for evaluating biomarkers of aging were offered: (i) a significant cross-sectional correlation with age; (ii) a significant longitudinal change with age consistent with the cross-sectional correlation; (iii) significant stability of individual differences; and (iv) a rate of age-related change proportional to differences in life span among related species. In the present study, we applied these criteria to human data to identify candidate biomarkers of aging and to construct a BAS based on this analysis. We could not apply the 4th criterion to the current analysis because of the lack of certain data (e.g., FEV_1/Ht^2) from other species. By following a stepwise selection process applying the first three criteria, we identified five variables (FEV_1/Ht^2, SBP, HCT, ALBU, and BUN) as candidate biomarkers of aging in the present study.

The selected biomarkers of aging represent a variety of health measures that have proven predictive of age-related

disease and mortality. Kannel and McGee (25) and Weiss and colleagues (26) demonstrated that a low FEV_1 was associated with a substantial excess risk for cardiovascular disease, and also suggested that the rate of decline in FEV_1 was a predictor of total mortality among smokers. Hypertension is often a silent cardiovascular risk factor. The age-related increase in SBP can reflect many factors including atherosclerosis-induced reduction in arterial elasticity (27). Blazer and colleagues (28) suggested in a 6-year longitudinal study that, among older adults, there was a significant relationship overall between the SBP and mortality. Serum albumin is a crude indicator of nutritional status and shows a clear and significant decrease with advancing age (29,30). Furthermore, a combined measure of serum albumin and physical disability revealed a marked increase in mortality risk (31,32). Blood examination values such as RBC, HB, and HCT decrease as age advances, and were helpful to evaluate the effect of physiological aging in centenarians (33,34). Avorn and Gurwitz (35) found that BUN and serum CREAT levels were useful markers of the glomerular filtration rate and increased with age in older people. We consider, based on observations in the current study, that these physiological variables selected as biomarkers of aging reflect the general condition of an individual's health, emerging from the functional state of vital organs and physiological parameters closely related to the maintenance of life.

Further evidence of the validity of the selected measures was obtained when the five candidate biomarkers were submitted to a PCA. The emerging candidate biomarkers of aging were significantly related and appeared to reflect, at least statistically, an underlying aging process. With chronological age entered into the current analysis, the results revealed that one principal component related to chronological age plus the five candidate biomarkers could account for 42% of the total variance in the sample of healthy men. Thus, we obtained evidence that the five candidate biomarkers expressed substantial covariance along one principal component related to chronological age. Such evidence has been considered essential for demonstrating an underlying unitary or global aging process (5,6,18,36). A previous factor analysis of this data set also confirmed the existence of a general aging factor (19).

Following the confirmation of the underlying relationship among the five candidate biomarkers, we constructed a BAS for each individual by using the weighted loadings onto the factor score derived from the PCA. The calculation of the BAS followed the procedures previously applied in our analyses of aging in rhesus monkeys using measures of blood chemistry and hematology (17,18). Several analyses were then conducted to support the underlying reliability and validity of the BAS as derived for humans in the current study. First, we noted that the individual BAS values were scattered relatively symmetrically above and below the regression line for chronological age. Second, we demonstrated that individual BAS values showed a high longitudinal stability, as evidence of the predictive validity of this index. Third, we investigated age-related change in the BAS across three age groups (<45, 45–64, and ≥ 65 years of age) to assess whether the rate of change differed with advancing age. We found that the aging rates in men of different ages represented by the BAS were not constant, but rather followed an exponential curve. The rate of change in the BAS was 1.8 times higher in the old group than in the young group.

Individual differences are a hallmark of aging. It is clear from Figure 6 that individual differences are characterized by three factors: age, peak functional capacity, and aging rate. There is no doubt that individuals could have genetically determined differences in their peak functional capacities, and also have environmentally different rates of biological aging. However, a direct measure of the genetic and environmental mechanisms that determine the rate of these changes is not available. Thus, we tried to estimate the effects of environmental factors on the BAS, based on the data obtained from the self-administered questionnaire. In the present study, a multiple logistic regression analysis was used to analyze the effects of environmental factors such as disease, occupation, smoking status, drinking habits, and level of exercise on high aging rates and high peak functional capacities in terms of the BAS. Three factors (chronological age, smoking status, and level of exercise) had a close relationship with individual aging rates. However, these environmental factors did not have any close relationships with an individual's peak functional capacity. Therefore, an individual's aging rate may be widely affected by environmental factors such as smoking status and level of exercise. From these results, it was estimated that an individual's aging rate was mainly influenced by environmental factors, but an individual's peak functional capacity was almost independent of environmental factors. It seems that a genetic factor acts on peak functional capacity. The concept of biological age is based on the idea that the aging rate of representatives of one species varies over certain ranges. Therefore, the biological age of population members whose aging rates are slower or quicker is respectively lower or higher than their chronological age. A deviation from a regression line of the BAS values on age does not necessarily reflect an individual's aging rate. Rather, it might reflect an individual's genetic characteristics. However, because the explanatory variables of environmental factors are crudely assessed as simple dichotomies, whether the above-mentioned explanation is true or whether another one is required cannot be known until further study is done.

The validity of the candidate biomarkers of aging and the BAS derived in the present study could be further supported by demonstrating the ability to predict life span among individuals. However, as humans are a long-lived species, it is difficult to follow the life span of a person until death. We therefore decided to rely on a functional assessment, the BAS. It is questionable whether the estimated BAS can be used to truly estimate life expectancy. Short and colleagues (6) suggested that an alternative measure to longevity is the rate of aging. Thus, we considered that the measurement of BAS was also useful as an alternative to the use of survival measures.

Conclusion

The five biomarkers of aging identified in the current study are physiologically relevant and are generally easy to obtain in clinical studies. Individual BAS values showed

high longitudinal stability of age-related changes and accelerated change with age. We are not proposing the current BAS as constructed to be the final useful product emerging from this enterprise. Indeed, a much wider database with additional variables, collected at other levels of biological organization (from molecular to behavioral), could be examined to identify additional candidate biomarkers of aging. In addition, other populations need to be evaluated to determine the robustness of the selected biomarkers and the BAS derived in the current study. Also, as the data set was highly selected (e.g., participants were excluded for the presence of many diseases) and ends with the oldest individual at 77 years of age, the applicability of biomarkers developed can only be used in narrowly defined subsets. Furthermore, as is evident from Table 6, an individual's aging rate may be widely affected by smoking status and level of exercise. In the current study, smokers (24 of 86 healthy participants) and exercisers (28 of 86 healthy participants) were included in the data set. The data should be reanalyzed with smokers and exercisers removed. This is a subject for future study.

Acknowledgments

We express our sincere gratitude to Dr. Donald K. Ingram, Laboratory of Experimental Gerontology, Gerontology Research Center, National Institute on Aging, National Institutes of Health, for his valuable advice and criticism.

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Received March 27, 2006
Accepted June 5, 2007
Decision Editor: Huber R. Warner, PhD