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Further Evaluation of the Basic Nature of the Human Biological Aging Process Based on a Factor Analysis of Age-Related Physiological Variables

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This study aimed to reexamine whether there exists a primary aging process that controls the rate of aging in a number of different functions. Eighty-six adult males who successively received a 2-day routine health checkup test for 7 years from 1992 to 1998 at the Kyoto Red Cross Hospital were selected as subjects. Nine candidate biomarkers of aging were selected from the 25 physiological variables based on the investigation of age-related changes. A principal factor analysis was applied to the partial correlation matrix for 9 selected biomarkers calculated by controlling for age. Furthermore, a confirmatory factor analysis in testing first- and second-order factor models was applied to the covariance matrix for 9 biomarkers. The results of these factor analyses revealed that there existed one general factor and three system-specific factors. Therefore, biological age changes can be viewed as a time-dependent complex integration of the primary and secondary aging processes.

RECENTLY, studies on the slowing of aging in humans by calorie restriction, drug treatment, and exercise regimens have increased. The purpose of these interventions is to increase the well-being of the elderly population by preventing age-related chronic diseases and to help maintain a vigorous, active, and productive life until death (1,2). However, a standardized method for examining the effectiveness of these interventions, such as a biological aging measurement system, has yet to be established (3,4).

Biological aging is defined as a process or group of processes that result in the progressive decrement of viability of the organism with advancing age (5–7). Whether a person is biologically younger or older suggests the quality of functioning organ systems and reflects the probability of death. Therefore, biological age can be viewed as an objective measure for the assessment of one's biological vigor, which declines with advancing age (5–8).

It was suggested that a biological age will allow us to determine the rate of aging within an individual and will also aid in monitoring the influence of environmental factors on the aging processes (4,9). However, the concept of biological age or functional age has met with considerable controversy regarding its validity and utility (4,10–13). This controversy stems in part from early attempts to define a single index of "biological or functional age" for humans on the basis of multiple regression analyses derived from cross-sectional studies (see, e.g., 14–16). Costa and McCrae (17,18) reported that the evidence from such studies suggests that these analyses do not provide better information about biological age than does chronological age itself. Furthermore, some gerontologists cast considerable doubt on the existence of unitary aging indices that control the rate of aging in different organs and functions, because most physiological variables do not necessarily change with age in the same way or at the same rate (4,17,19). Although human development and maturation follow a fixed path that is based on time from either conception or birth, there is little evidence for the existence of the coordinated processes governing the rate of human aging.

In a previous study (20), we investigated whether there existed a primary aging process that controls the rate of aging in a number of different functions based on a hierarchical factor solution of age-related physiological variables. The findings of that study suggested the possibility that biological age changes could be viewed as a time-dependent complex integration of the primary and secondary processes. However, only a relatively small percentage of the parameter's variance was due to primary aging (10.3%). These results were also found in a cross-sectional study that estimated the rate of aging from relative standing on a set of functions. A cross-sectional study based on data obtained at any one point in time cannot indicate the age changes directly (19,21,22). Therefore, we need to reexamine these results by using longitudinal data.

The purpose of this study was to reexamine, using factor analysis, whether a primary aging process existed that controls the rate of aging in a number of different physiological functions, based on the data for physical examinations and the routine battery of hematology and blood chemistry tests in a 7-year longitudinal study of healthy Japanese men.

Methods

Subjects

Among approximately 18,000 Japanese adult men who received a routine health checkup test from 1992 to 1998 at the Kyoto Second Red Cross Hospital, 122 adult males who

Table 1. Clinical Criteria for Normality

Criteria	Value	Criteria	Value
Obesity index (%)*	<20	HCT (%)	40–52
Blood pressure	<160/95	Urine SG	1.01–1.025
(mm Hg)		Urine pH	5.0–8.0
ECG	Normal	Total protein (g/dl)	6.6–8.4
	ECG and	Albumin (g/dl)	3.5–5.5
	rhythm	A/G ratio	1.0–2.0
X-rays	Normal	Total chol. (mg/dl)	130–260
Chest and digestive		HDL chol. (mg/dl)	40–120
system		Triglyceride (mg/dl)	40–150
%FVC	>80	Urea nitrogen (mg/dl)	8.0–23.0
$\%FEV_1$	>70	Creatine (mg/dl)	0.7–1.5
Blood glucose (mg/dl)	<110	Uric acid (mg/dl)	4.0–7.5
OGTT, 1 h (mg/dl)	<140	Total bilirubin (mg/dl)	0.2–1.2
OGTT, 2 h (mg/dl)	<120	GOT (Iu/l)	10–35
Precipitation of blood,	<10	GPT (Iu/l)	5–35
1 h (mm)		LDH (mg/dl)	40–120
RBC (10^4/mm^3)	440–560	ALP (Iu/l)	50–140
WBC (10^2/mm^3)	4000–9000	Ca (mEq/l)	4–5
HB (g/dl)	13–16	Na (mEq/l)	138–148

Notes: ECG = electrocardiogram; FVC = forced vital capacity; FEV_1 = forced expiratory volume in 1 second; OGTT = oral glucose tolerance test; RBC and WBC = red and white blood cell counts; HB = hemoglobin; HCT = hematocrit; SG = specific gravity; A/G = albumin to globulin; HDL = high-density lipoprotein; GOT = glutamate oxaloacetate transaminase; GPT = glutamic pyruvic transminase; LDH = lactic dehydrogenase; ALP = alkaline phosphatase; Ca = calcium; Na = sodium. The obesity index was calculated from the following equations: Standard weight (kg) = [Height (cm) − 100] × 0.9; obesity index = [{measured weight (kg) − standard weight (kg)}/standard weight (kg)] × 100.

successively received a 2-day routine health checkup test once a year (April to May) for 7 years from 1992 to 1998 were randomly selected as subjects. Each subject's past and present health status, work history, social and dietary habits, and the like were determined from the medical questionnaire. Informed consent was obtained from all subjects. Of the 122 subjects, 36 were excluded as subjects with abnormal measurement values. Some had diseases such as critical hypertension (7 men), diabetes (3 men), asthma or chronic cough (5 men), or excessive obesity (3 men); others showed "stress" effects for blood pressure (7 men), in which the stress of the testing environment had an effect on blood pressure, and there was a "practice" effect for pulmonary functions (11 men), in which subjects respond better in later trials than in earlier trials because they learn the appropriate responses during the 7-year observation period (21,22). Finally, 86 adult males were selected as subjects in this study. Most of them were judged as healthy on the basis of clinical criteria for normality of subject, which was set by the Japanese Red Cross Hospital (Table 1). However, several elderly men with hypertension, diabetes, and hyperlipemia tendencies were included in the subject group. We well recognize that aging is a normal process, not a disease. In case of old people, it is difficult to distinguish between normal and abnormal aging, because as we grow older, normal age-related decrements in vital organs do produce increased vulnerability to pathological change. Most subjects resided in Kyoto City. Their occupations were as follows: managers (11 persons), salesmen (18 persons), researchers and engineers (5 persons), storekeepers (10 persons), teachers (5 persons), unemployed (16 persons), and various others (21 persons). The age range of subjects in several age cohorts at the beginning of this study were from 31 to 77 years, with a mean age of 51.2 years.

Test Items and Test Procedures

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, we used the following 25 items tested in the routine checkup in this study.

There were results of the cardiorespiratory function test: 1, systolic blood pressure and 2, diastolic blood pressure (SBP and DBP, both in millimeters of mercury); 3, forced vital capacity (FVC, in liters); and 4, forced expiratory volume in 1.0 second (FEV_1, in liters). There were results of the blood examination: 5, white blood cell count and 6, red blood cell count (WBC and RBC, in 10^2 or 10^4 per cubic millimeter); 7, hemoglobin concentration (HB, in grams per decaliter); and 8, hematocrit (HCT, in percent). There were results of the biochemical examination of serum: 9, total protein, 10, albumin, and 11, globulin (TPRO, ALBU, and GLOB, all in grams per decaliter); 12, ratio of albumin to globulin (A/G ratio); 13, total bilirubin (TBILI, in milligrams per decaliter); and 14, alkaline phosphatase, 15, glutamate oxaloacetate transaminase, 16, glutamic pyruvic transaminase, and 17, lactic dehydrogenase (ALK, GOT, GPT, and LDH, all in international units per liter). There were also 18, blood urea nitrogen, 19, creatine, 20, uric acid, and 21, calcium (BUN, CREAT, URIC, and CALC, all in milligrams per decaliter). Finally, there were 22, total cholesterol, 23, triglyceride, 24, high-density lipoprotein cholesterol, and 25, blood glucose (TC, TG, HDL-C, and GLU, all in milligrams per decaliter).

Of the aforementioned tests, pulmonary function (FEV_1 and FVC) was measured by use of an electric spirometer (System-9, Minato Co. Ltd., Osaka, Japan) three times while subjects were standing, and the best record was used. Whether or not each maneuver was acceptable was assessed by the following criteria: starting without hesitation, apparent maximal effort, and smooth continuous exhalation without cough. Reproducibility was judged by the criteria of the American Thoracic Society (23). Blood pressure (SBP and DBP) was measured manually with a sphygmomanometer after subjects took 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 with a Hitachi Automatic Analyzer (Model-7150, Tokyo, Japan). The hematological measurements were made on a Symex Automatic Blood Analyzer (E-4000, Tokyo, Japan).

Statistical Analysis

The mathematical function that describes the age-related changes of physiological variables was mainly calculated by linear regression. As a way to discern the underlying

structure of intercorrelation among the selected biomarkers, the following two kinds of factor analyses were applied: first, a principal component analysis; second, a linear structural relation (LISREL) approach to confirmatory factor analysis (CFA) in testing first- and second-order factor models (24). The analysis of first-order factors is an application of LISREL's measurement model, and the analysis of second-order factors is an application of LISREL's structural equation model. The validity of this model (CFA) was tested on the basis of the following three goodness-of-fit indexes: goodness of fit index (GFI); adjusted GFI (AGFI); and root mean square error of approximation (RMSEA). The values of GFI and AGFI range from 0 to 1.0. The acceptance of the model is generally determined at a value greater than 0.9, and there exists the relation of AGFI $\leqslant$ GFI. The value of RMSEA approaches zero if the observed data are nearly equal to the data reproduced from the model. This analysis was performed with an Amos application computer program (25). All computations except for the analysis of covariance structures were made with computer programs in the Statistical Package for Social Sciences (26).

Results

Selection of Candidate Biomarkers of Aging

Before any factor analytical solutions were applied, the validity of the data as biomarkers of aging had to be examined in detail. A biomarker of aging refers to a biological parameter intended as a quantitative measure of the rate of aging that is more accurate than chronological age (27). However, biomarker research remains a controversial area within gerontology. Little consensus has been achieved concerning definitions, terminology, selection, and validation processes. From the primate study initiated in 1987 at the National Institute on Aging, the following criteria for defining a biomarker of aging have been offered: (a) significant cross-sectional correlation with age; (b) significant longitudinal change in the same direction as the cross-sectional correlation; (c) significant stability of individual differences; and (d) rate of age-related change proportional to differences in life span among related species. In the present study, these criteria (except for the fourth) were applied to the selection of candidate biomarkers of aging. Table 2 shows the set of correlations used to guide the first three steps of the selection process to identify a set of biomarkers of aging.

Step 1: Cross-sectional analysis.—Candidate biomarkers of aging are expected to show evidence of change with the passage of time. To identify the degree of relation between each variable and chronological age, we first calculated the mean of the 7 observations for each variable and the chronological age of each subject. We then calculated the correlation of mean variable with mean age, using all the subjects ($N = 86$). On the basis of this criterion, we identified the following 11 statistical significant candidate variables: FVC, FEV_1, SBP, RBC, HB, HCT, ALBU, A/G ratio, BUN, CREAT, and CALC ($p < .05$). The highest correlation with chronological age was observed for FEV_1 (-0.75). Relatively high correlations were observed for FVC (-0.60) and ALBU (-0.49).

Table 2. Summary of Correlation Coefficients for 86 Healthy Adult Men

Variable	Cross-Sectional Analysis ($n = 86$)	Longitudinal Analysis ($n = 86$)	Stability Analysis ($n = 86$)
1. FVC	−0.602**	−0.478**	0.927**
2. FEV_1	−0.747**	−0.596**	0.928**
3. SBP	0.395**	0.580**	0.807**
4. DBP	0.179	0.405**	0.797**
5. WBC	−0.069	−0.115	0.747**
6. RBC	−0.344**	−0.367**	0.869**
7. Hemoglobin	−0.232*	−0.229*	0.832**
8. Hematocrit	−0.277**	−0.435**	0.815**
9. TPRO	−0.176	0.019	0.650**
10. Albumin	−0.485**	−0.310**	0.817**
11. Globulin	0.152	0.112	0.657**
12. A/G ratio	−0.261*	−0.222*	0.685**
13. TBILI	0.011	−0.040	0.640**
14. ALK	0.183	−0.333**	0.872**
15. GOT	0.130	0.101	0.597**
16. GPT	−0.080	−0.037	0.697**
17. LDH	0.214	−0.245*	0.775**
18. BUN	0.404**	0.251*	0.703**
19. Creatine	0.296*	0.180	0.845**
20. Uric acid	−0.179	0.009	0.822**
21. Calcium	−0.229*	−0.173	0.680**
22. Total chol.	0.208	−0.165	0.644**
23. Triglyceride	−0.141	0.003	0.685**
24. HDL-C	0.168	0.179	0.841**
25. Blood glucose	0.173	0.129	0.849**

Notes: Correlation coefficients were obtained from cross-sectional, longitudinal, and stability analyses. FVC = forced vital capacity; FEV_1 = forced expiratory volume in 1 second; SBP and DBP = systolic and diastolic blood pressure; WBC and RBC = white and red blood cell (count); TPRO = total protein; A/G = albumin to globulin; TBILI = total bilirubin; ALK = alkaline phosphatase; GOT = glutamate oxaloacetate transminase; GPT = glutamic pyruvic transminase; LDH = lactic dehydrogenase; BUN = blood urea nitrogen; HDL-C = high-density liproprotein cholesterol.

$^*p < .05$; $^{**}p < .01$.

Step 2: Longitudinal analysis.—To identify the degree of longitudinal changes in each variable, we first transformed the measurement variable and chronological age of each subject across 7 years to z scores to standardize the scales. We then calculated correlations between chronological age and the values for each subject, the so-called standardized slope of the regression line for the variable of an individual. Using Fisher's transformation of r to z and z to r, we then calculated the means of individual r of all the subjects. On the basis of this analysis, we identified the following 12 significant potential biomarkers: SBP, DBP, FVC, FEV_1, RBC, HB, HCT, ALBU, A/G ratio, ALK, LDH, and BUN. The highest correlation with chronological age was observed for FEV_1 (-0.60), followed by SBP (0.58). Relatively high correlations with chronological age were observed for FVC (-0.48), HCT (-0.44), and DBP (0.41).

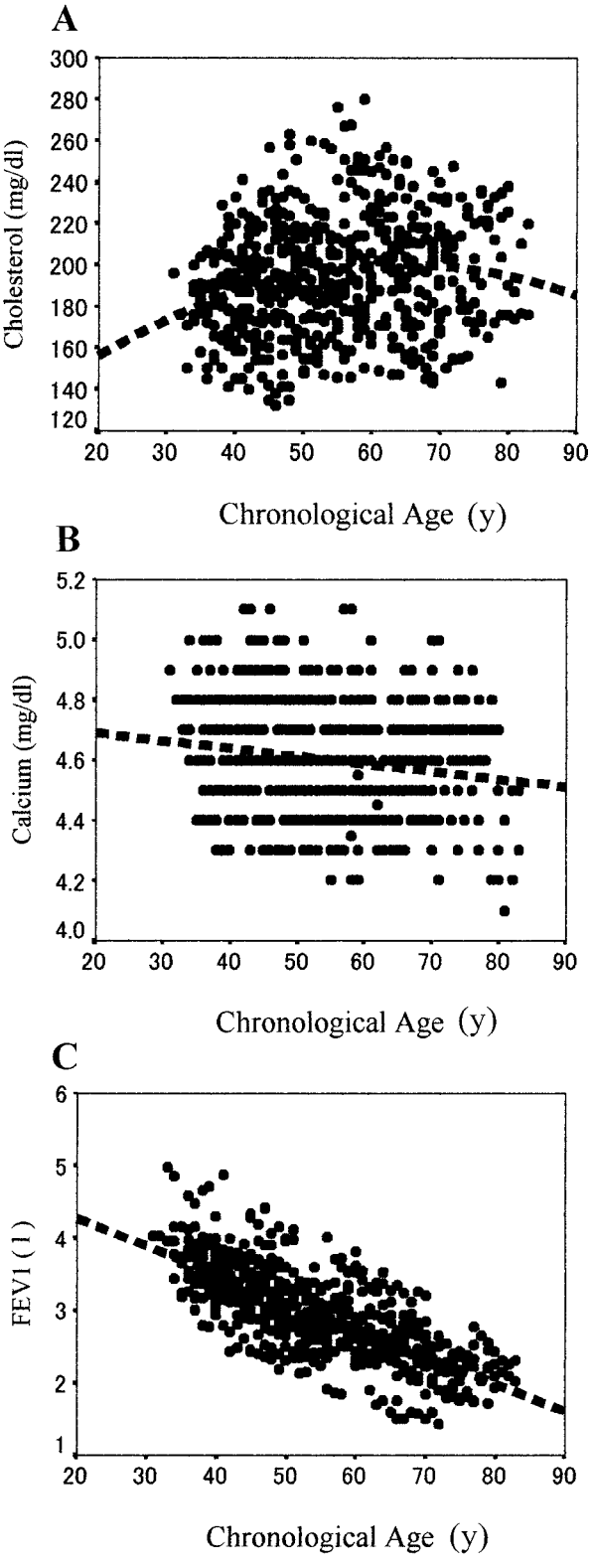

Figure 1. Three types of age-related changes: **A**, cholesterol, **B**, calcium, and **C**, forced expiratory volume in 1 second (FEV_1) levels.

Step 3: Stability analysis.—Next, we examined the degree of longitudinal stability of individual differences in selected biomarkers. For this analysis, we evaluated the interyear reliability of the annual values for each variable. Specifically, we calculated correlations 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, and 1993 and 1994, across all ages within subjects. Then we applied a partial correlation analysis by using chronological age as the covariate to eliminate the effects of age. To calculate a mean value of the partial correlation coefficients across the 7 years, we applied the Fischer transformation of r to z and z to r. All the variables used in this study showed significant stability ($p < .01$).

On the basis of the aforementioned three types of analyses, we selected potential biomarkers of aging. Namely, 11 variables from the cross-sectional analysis and 12 variables from the longitudinal analysis were tentatively selected as potential biomarkers of aging. All the variables selected in this step showed significant stability ($p < .01$), with the magnitude of correlation greater than 0.7. These variables were considered to show age-related changes. Half of all variables did not show age-related changes, but some showed a possibility that, after 70 years of age, their measurement values began to show age-related change. Figure 1 presents three types of age-related changes inferred from the physiological variables used in this study. Figure 1A (cholesterol) shows no significant cross-sectional correlation with chronological age. The ALK, LDH and TG variables were observed as variables of this type, and these variables showed disagreement between cross-sectional and longitudinal age-related changes. Figure 1B (calcium) shows no significant cross-sectional correlation with chronological age until older age, but thereafter it shows an age-related change. The URIC, CREAT, and TBILI were observed as variables of this type. FEV_1 showed a significant cross-sectional correlation with chronological age (Figure 1C). The variables that showed significant age-related changes in both cross-sectional and longitudinal analyses were observed as variables of this type. In this study, potential biomarkers of aging were selected from the variables of this type (Figure 1C).

We finally identified the following 9 potential biomarkers of aging: FVC, FEV_1, SBP, RBC, HB, HCT, ALBU, A/G ratio, and BUN. The two variables ALK and LDH that showed significant age-related changes in the longitudinal analyses showed disagreement for the direction of age-related changes in parameters with those variables selected in the cross-sectional analysis. The DBP did not show a significant correlation in the cross-sectional analysis. These variables were eliminated from the candidate biomarkers.

Factor Analysis of the Selected Biomarkers

Table 3 shows the mean and standard deviation (*SD*) and the correlation matrix of 9 biomarkers of aging calculated from the 7-year longitudinal data of 86 healthy adult males. The information in this table is based on the slope of the regression line taken as the measure of the rate change of the biomarker. For each subject and each variable, the line of best fit was calculated by linear regression, using the

Table 3. Mean and *SD* of 9 Physiological Variables and Correlation Coefficients Among Them

Variable	1	2	3	4	5	6	7	8	9
1. FVC (l)	1.000								
2. FEV_1 (l)	0.479	1.000							
3. SBP (mmHg)	−0.010	−0.111	1.000						
4. RBC ($10^4/mm^3$)	−0.109	0.058	0.139	1.000					
5. Hemoglobin (g/dl)	0.050	0.195	0.310	0.729	1.000				
6. Hematocrit (%)	0.043	0.224	0.191	0.822	0.881	1.000			
7. Albumin (g/dl)	0.182	0.172	−0.354	0.011	0.067	0.071	1.000		
8. A/G ratio	0.059	0.090	−0.350	−0.236	−0.197	−0.249	0.571	1.000	
9. BUN (mg/dl)	0.068	0.041	0.145	−0.117	−0.019	−0.012	−0.071	−0.075	1.000
Mean	−0.043	−0.048	2.212	−2.831	−0.041	−0.314	−0.014	−0.005	0.110
SD	0.045	0.035	1.870	4.140	0.120	0.370	0.035	0.030	0.450

Notes: These statistics were calculated by using the data for the slopes of regression lines on chronological age in 86 healthy male subjects. FVC = forced vital capacity; FEV_1 = forced expiratory volume in 1 second; SBP = systolic blood pressure; RBC = red blood cell (count); A/G = albumin to globulin; BUN = blood urea nitrogen. R = .217 required for $p < .05$.

subject's age at the time measurements were taken as the independent variable. The mathematical function that best describes the change in an attribute over the entire life span is undoubtedly not linear, but change can be approximated by a linear function if the time span is not great. Therefore, the slope of the line was taken as the measure of the rate of change of that variable.

As a way to discern the underlying structure of intercorrelations among the 9 selected biomarkers, first of all, a principal factor analysis was applied to the correlation matrix of those variables (Table 4). Three principal factors with eigenvalues (the variance of component) of >1.0 were obtained, following the recommendations of Guttman (28). However, the communality of BUN after three factors were extracted was too low (0.270). This means that the extraction of factors is insufficient. Thus we tried to extract four factors (Table 5). Our factor model explained 79.2% of the total variance of all variables. The remaining 20.8% is called *uniqueness*, which consists of the error variance and specificity of each variable. The first principal factor was significantly loaded with 5 out of 9 biomarkers, and it explained approximately 32% of the total variance of all variables. The three variables RBC, HB, and HCT especially showed extremely high factor loadings (0.864, 0.913, and 0.935, respectively). Thus this factor was interpreted as the "hematology factor," but it leaves the possibility of the existence of a primary aging factor. The second factor showed high factor loadings with the two variables ALBU (0.794) and A/G ratio (0.632), so this factor was interpreted as the "protein metabolism factor." The third factor was interpreted as the "pulmonary function factor," because the two variables FVC (0.700) and FEV_1 (0.545) showed high factor loadings. The fourth factor was interpreted as the "renal function factor," because the BUN (0.807) showed an extremely high factor loading. These factors represent system-specific factors.

Table 6 shows a partial correlation matrix among 9 candidate biomarkers of aging calculated by controlling for age. It is likely that there are correlated changes in these functions represented by these 9 biomarkers, because they all deteriorate more rapidly in old age. Thus individual chronological age has to be controlled. A principal component analysis was again applied to this partial correlation matrix (Table 7). In this factor analysis, four factors were extracted to compare this with the previous result in Table 5. This factor model explained 62.0% of the total variance of all variables. The difference of total variance between this model and the previous model is 17.2%, suggesting that this difference might be due to the effect of aging. The first principal factor was significantly loaded with 5 out of 9 biomarkers in this model, too, and explained approximately 30% of the total variance of all variables. There were only three factors that explain the total variance more than 10%: Factor 1 as hematology factor, Factor 2 as protein metabolic factor, and Factor 3 as pulmonary function factor. It is expected that the first unrotated principal factor might represent a primary aging factor, because this factor explained approximately 50% of the total variance of the extracted four factors.

Analysis of a Hierarchical Factor Model by LISREL Analysis

To investigate the existence of a primary aging factor from a different angle, we applied a CFA in testing first- and second-order factor models to a covariance matrix for

Table 4. Principal Component Analysis for 9 Selected Biomarkers (Without a Factor Rotation)

Biomarker	Factor			Communality (h^2)
	F1	F2	F3	
1. FVC		0.481**	0.700**	0.722
2. FEV_1	0.185	0.586**	0.545**	0.675
3. SBP	0.406**	−0.497**	0.319**	0.513
4. RBC	0.863**		−0.297**	0.843
5. Hemoglobin	0.913**	0.185		0.871
6. Hematocrit	0.935**	0.210		0.925
7. Albumin	−0.118	0.794**	−0.250*	0.707
8. A/G ratio	−0.435**	0.632**	−0.266*	0.660
9. BUN		−0.147	0.498**	0.270
Eigenvalue	2.855	1.961	1.369	6.185
%Total variance	31.72	21.79	15.21	68.74

Notes: Factor loadings less than ±0.1 were omitted. This analysis was applied to a correlation matrix for 9 biomarkers. FVC = forced vital capacity; FEV_1 = forced expiratory volume in 1 second; SBP = systolic blood pressure; RBC = red blood cell (count); A/G = albumin to globulin; BUN = blood urea nitrogen.

*$p < .05$; **$p < .01$.

Table 5. Principal Component Analysis for 9 Selected Biomarkers (Without a Factor Rotation)

Biomarker	Factor				Communality (h^2)
	F1	F2	F3	F4	
1. FVC		0.481**	0.700**	−0.218	0.770
2. FEV_1	0.185	0.586**	0.545**	−0.245*	0.735
3. SBP	0.406**	−0.497**	0.319**	0.125	0.529
4. RBC	0.863**		−0.297**		0.843
5. Hemoglobin	0.913**	0.185			0.880
6. Hematocrit	0.935**	0.210			0.930
7. Albumin	−0.118	0.794**	−0.250*	0.282*	0.787
8. A/G ratio	−0.435**	0.632**	−0.266*	0.272*	0.734
9. BUN		−0.147	0.498**	0.807**	0.920
Eigenvalue	2.855	1.961	1.369	0.942	7.127
%Total variance	31.72	21.79	15.21	10.46	79.19

Notes: Factor loadings less than ±0.1 were omitted. This analysis was applied to a correlation matrix for 9 biomarkers. FVC = forced vital capacity; FEV_1 = forced expiratory volume in 1 second; SBP = systolic blood pressure; RBC = red blood cell (count); A/G = albumin to globulin; BUN = blood urea nitrogen.

*$p < .05$; **$p < .01$.

the 9 biomarkers. Data are based on the 7-year longitudinal data ($N = 602$) of 86 subjects. In this model, one general factor was hypothesized to explain the covariance among three system-specific factors based on the results of the aforementioned exploratory factor analysis (Table 7). In addition, all paths connecting the error variables (e1–e9) and disturbance variables (d1–d3) were fixed to unity, and one loading for each factor was also fixed to unity to ensure the identification of this model. Figure 2 shows the results of this model estimated by a LISREL analysis. For the determination coefficient of the whole model, the GFI was 0.957, the AGFI was 0.919, and RMSEA was 0.080. This hierarchical factor model of the 9 biomarkers was statistically valid from the degrees of model-fitting indicators. From these results, it was inferred that there existed one general factor that controls sub-system-specific factors and three system-specific factors (pulmonary function factor, hematology factor, and protein metabolism factor).

Discussion

An important goal in the field of gerontology is to assess the complexity of aging processes or the processes governing the rate of aging. Unfortunately, we do not yet know if a primary aging process exists. If the laws are known, an objective measure of assessing one's biological age can be obtained, and the confusion concerning the measurement of biological aging that stems from the lack of agreement on the basic nature of the aging process can be resolved. In the present study, primary aging and secondary aging were defined, following of the definition of Hofecker and colleagues (7), as follows. Primary aging is related to the biological processes of decline rooted in heredity, and consequently appears to be very similar in most or all body systems; secondary aging reflects the secondary *process* of aging, which includes deleterious consequences of primary aging as well as compensatory changes to maintain homeostasis. However, these aging processes are not recognizable in all people, and those that are present do not appear at the same time or progress at the same rate. We have to estimate those processes through multivariate statistical analyses such as factor analysis.

Costa and McCrae (17,18) reported that if aging was a single process, a factor analysis of change scores from one time to another on different physiological tests administered to subjects of different ages should show a single general factor common to many physiological tests. Namely, a general aging factor has to be able to account for all or most the observable changes that occur with age. Several studies have applied factor analysis to the results of a diversified battery to identify the existence of a general aging factor (7,29–31). Hofecker and colleagues (7) reported that 23 age parameters of rats were grouped into six factors, and the first factor, which was loaded significantly with 17 of the 23 parameters and accounted for 41% (relative importance of the factors: 67%) of total variance of all variables, could be interpreted as a general aging factor. However, in human studies this method did not confirm a hypothesis on the organization of multicellular aging, because only a small percentage of the parameter's variance was due to aging (29–31). Furthermore, the results of their factor analysis do not necessarily reflect age change because their studies were done cross-sectionally, rather than longitudinally.

Costa and McCrae (17) examined a factor analysis with the varimax rotation of changed scores over two successive 5-year periods for a variety of biomedical, anthropometric, and psychosocial variables. Twenty factors with eigenvalues greater than 1.0 were obtained, but a general aging factor

Table 6. Partial Correlation Coefficients Among 9 Candidate Biomarkers of Aging Calculated by Controlling for Age

Biomarker	1	2	3	4	5	6	7	8	9
1. FVC (l)	1.000								
2. FEV_1 (l)	0.466	1.000							
3. SBP (mmHg)	0.009	−0.100	1.000						
4. RBC ($104/mm^3$)	−0.101	0.076	0.133	1.000					
5. Hemoglobin (g/dl)	0.046	0.180	0.320	0.742	1.000				
6. Hematocrit (%)	0.033	0.214	0.197	0.833	0.880	1.000			
7. Albumin (g/dl)	0.162	0.142	−0.347	0.023	0.052	0.059	1.000		
8. A/G ratio	0.039	0.059	−0.343	−0.228	−0.217	−0.267	0.557	1.000	
9. BUN (mg/dl)	0.089	0.062	0.135	−0.129	−0.009	−0.009	−0.046	−0.052	1.000

Notes: These statistics were calculated by using the data for the slopes of regression lines on chronological age in 86 healthy men. FVC = forced vital capacity; FEV_1 = forced expiratory volume in 1 second; SBP = systolic blood pressure; RBC = red blood cell (count); A/G = albumin to globulin; BUN = blood urea nitrogen. R = .217 required for $p < .05$.

could not be extracted because even in the first factor, which showed the largest eigenvalues, its value was 2.58 (giving 5.5% of the total variance of all variables). The cause of this failure may be attributed to the wrong choice of parameters, the inhomogeneity of the sample, and the nonoptimal statistical approach. Especially a factor analysis with an orthogonal varimax rotation is not an adequate factor model to identify a general aging factor, because this analysis aims to obtain "a simple structure" to facilitate the interpretation of the extracted factor, and consequently much of the variance associated with the first factor was distributed to the other factors. Thus a hierarchical factor model should be used for this purpose. Furthermore, the biomedical, anthropometrical, and psychosocial variables used in their studies do not always show age-related change. The use of ambiguous variables will result in poor statistical correlations with chronological age. In general, the data for biomarkers of aging collect a great deal of unwanted information (32). Namely, each parameter's variance consists of genuine aging differences between individuals and differences not related to aging. It is necessary to examine the variables from this point of view. In the present study, we used only physiological data closely related to the vital organic functions to select potential biomarkers, and we also used healthy adult male subjects as far as possible to remove the effect of disease. Furthermore, as is evident from Figure 1, the variables that do not show any age-related change, such as cholesterol, were excluded in this study. If we expect to extract a general aging factor common to many physiological tests, the measured variables are expected to show evidence of change with the passage of time. Therefore, we first examined the age-related changes of all

Table 7. Principal Component Analysis for the 9 Selected Biomarkers (Without a Factor Rotation)

Biomarker	Factor				Communality (h^2)
	F1	F2	F3	F4	
1. FVC		0.307**	0.530**		0.383
2. FEV_1	0.185	0.445**	0.639**	−0.191	0.677
3. SBP	0.326**	−0.431**	0.176	0.200	0.499
4. RBC	0.844**		−0.245*	−0.189	0.814
5. Hemoglobin	0.903**	0.121		0.152	0.851
6. Hematocrit	0.954**	0.150			0.936
7. Albumin		0.873**	−0.269*	0.250*	0.904
8. A/G ratio	−0.349**	0.527**	−0.185		0.439
9. BUN			0.194	0.420**	0.439
Eigenvalue	2.708	1.561	0.927	0.387	5.583
%Total variance	30.09	17.34	10.30	4.31	62.04

Notes: Factor loadings less than ±0.1 were omitted. This analysis was applied to a partial correlation matrix for 9 biomarkers calculated by controlling for age. FVC = forced vital capacity; FEV_1 = forced expiratory volume in 1 second; SBP = systolic blood pressure; RBC = red blood cell (count); A/G = albumin to globulin; BUN = blood urea nitrogen.

$^{*}p < .05$; $^{**}p < .01$.

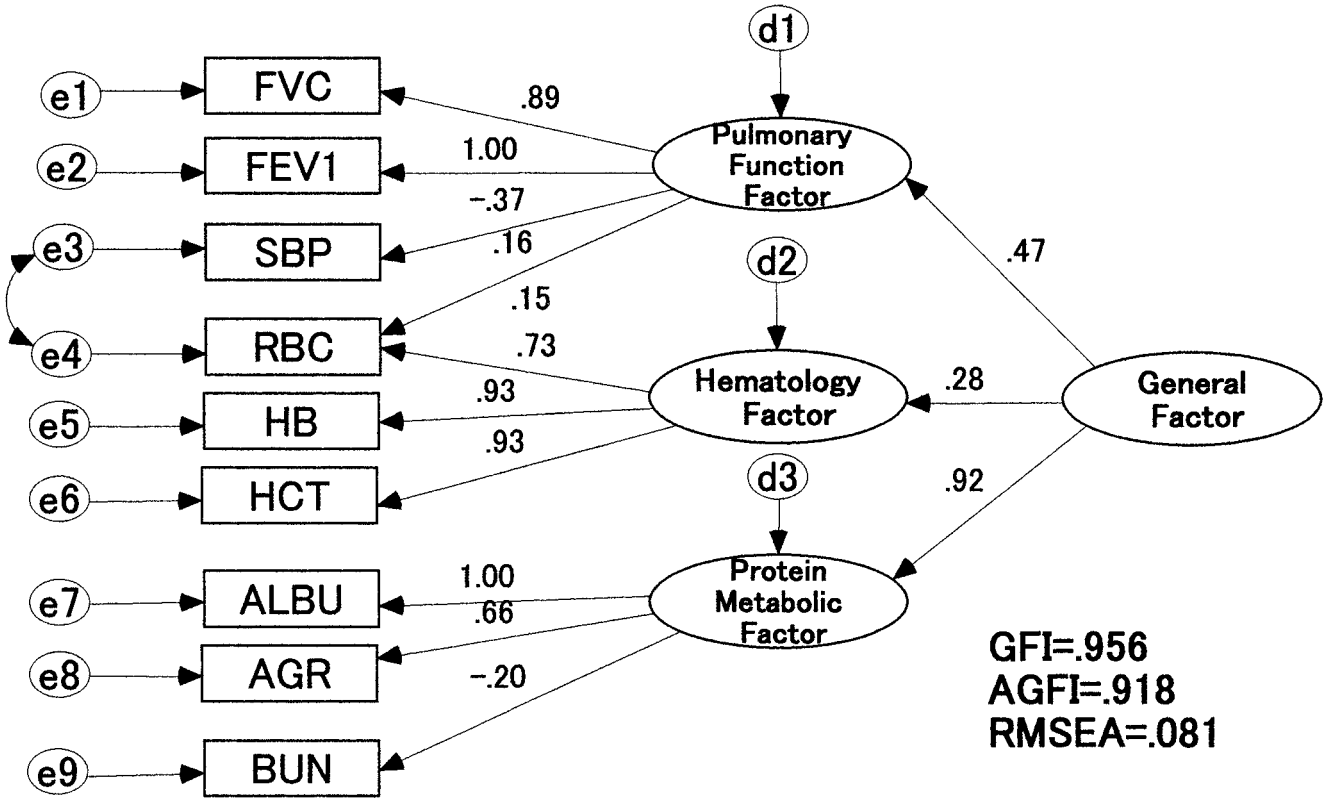

Figure 2. Linear structural relation approach to confirmatory factor analysis in testing first- and second-order factor models for the 9 candidate biomarkers of aging: FVC = forced vital capacity; FEV1 = forced expiratory volume in 1 second; SBP = systolic blood pressure; RBC = red blood cell (count); HB = hemoglobin concentration; HCT = hematocrit; ALBU = albumin; AGR = ratio of albumin to globulin; BUN = blood urea nitrogen; GFI = goodness-of-fit index; AGFI = adjusted GFI; and RMSEA, root mean square error of approximation.

physiological variables by means of both cross-sectional and longitudinal data analyses. As a result, the following 9 variables were selected as potential biomarkers of aging: FVC, FEV_1, SBP, RBC, HB, HCT, ALBU, A/G, and BUN.

As a way to investigate whether a general aging factor exists, first of all, a principal component analysis was applied to two kinds of correlation matrixes: first, a zero-order correlation matrix for 9 candidate biomarkers, and second, a partial correlation matrix for 9 candidate biomarkers calculated by controlling for age. The data used in this analysis are based on the slope of a linear regression. For each variable and each subject, we calculated a beta coefficient of regression line by using the subject's age as the independent variable in place of the change scores from one time to another proposed by Costa and McCrae (17). This approach aims to identify a general aging factor that controls the rate of aging in a number of biomarkers. Thus, the slope of the regression line was taken as the measure of the rate of change of the variable. The result of our factor analysis revealed that, in the first analysis, there were four system-specific factors, that is, the hematology factor, the protein metabolism factor, the pulmonary function factor, and the renal function factor, and in the second analysis there were three system-specific factors, that is, the hematology factor, the protein metabolism factor, and the pulmonary function factor. The percent contributions of the extracted factors to total variance were 79.2% for first analysis and 62.0% for second analysis. By partialling age out from the intercorrelation matrix for 9 biomarkers, this ratio dropped by 17.2%. As far as we examine the changes in the functions represented by the 9 selected biomarkers, they all deteriorate more rapidly in old age. Therefore, this difference might be due to the effect of aging.

Next we tried to examine the first principal component obtained from principal component analysis. We especially examined the result obtained from the partial correlation matrix calculated by controlling for age. Some psychologists who insist that there shall be a general factor point to the first centroid factor as evidence that there is such a factor (33). The first principal factor was significantly loaded with 5 out of 9 biomarkers and explained approximately 30% of the total variance of all variables. If such a variance is compared as a ratio of the variance to total variance of the extracted factors, this factor explains the variance of approximately 50%. The increment of this variance suggests that there remains the possibility of the existence of a primary aging factor. We examined this matter in more detail by using a confirmatory factor analysis in testing first- and second-order factor models. As is evident from Figure 2, the result of this analysis suggested that there existed one general factor that control sub-system-specific factors and three system-specific factors (i.e., the pulmonary function factor, the hematology factor, and the protein metabolic factor).

The pass coefficients (equivalent to correlation coefficients) to three factors that were drawn from a general factor were 0.46, 0.28, and 0.92, respectively. These results suggest that the relative importance of three factors to a general factor becomes the order of protein metabolic factor, pulmonary function factor, and hematology factor. This hierarchical factor solution of the 9 potential biomarkers proved the existence of primary aging factor inferred from the results of principal component analysis.

From the facts described herein, we may conclude that there exists one general aging factor that controls sub-system-specific factors and three system-specific aging factors. Therefore, biological age changes can be viewed as a time-dependent complex integration of primary and secondary aging processes. However, this research has some limitations concerning sample size and the choice of variables to study. After pathology was screened out, the end sample became 86 male subjects. Most factor analysts would consider this marginal at best. In addition, although biological age characterizes the general condition of an individual at a certain chronological age, which is marked by physical, mental, and social characteristics, the present study does not cover all these areas. We measured only such physical characteristics as anthropometric measurements, cardiorespiratory functions, hematology, and blood chemistry examinations. Therefore, this study only presents one possible approach for the investigation of the existence of primary aging.

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References

1. Anderson SF. An historical overview of geriatric medicine: definition and aims. In: Pathy MSJ, ed. *Principles and Practice of Geriatric Medicine*. London: J Wiley; 1985:7–13.
2. Shephard RJ. *Aging, Physical Activity, and Health*. Champaign, IL: Human Kinetics; 1997:303–324.
3. Ingram DK. Is aging measurable? In: Ludwig FC, ed. *Life Span Extension*. New York: Springer; 1991:18–42.
4. Masoro EJ. Physiological system markers of aging. *Exp Gerontol*. 1988;23:391–394.
5. Comfort A. Test-battery to measure aging-rate in man. *Lancet*. 1969;2: 1411–1415.
6. Ingram DK. Toward the behavioral assessment of biological aging in the laboratory mouse: concepts, terminology, and objectives. *Exp Aging Res*. 1983;9:225–238.
7. Hofecker G, Skalicky M, Kment A, Niedermuller H. Models of biological age of the rat. I. A factor model of age parameters. *Mech Ageing Dev*. 1980;14:345–359.
8. Dean W. *Biological Aging Measurement—Clinical Applications*. Los Angeles, CA: The Center for Bio-Gerontology; 1988:3–10.
9. Bowden DM, Short RA, Williamns DD. Constructing an instrument to measure the rate of aging in female pigtailed macaques (*Macaca nemestrina*). *J Gerontol Biol Sci*. 1990;45:B59–B66.
10. Adelman RC. Biomarkers of aging. *Exp Gerontol*. 1987;22:227–229.
11. Barker GT, Sprott RL. Biomarkers of aging. *Exp Gerontol*. 1988; 23:223–239.
12. Ingram DK. Key questions in developing biomarkers of aging. *Exp Gerontol*. 1988;23:429–434.
13. Ludwig FC, Smoke MW. The measurement of biological age. *Exp Aging Res*. 1980;6:497–521.
14. Hollingsworth JW, Hashizume A, Jablon S. Correlations between tests

of aging in Hiroshima subjects: an attempt to define "physiologic age." *Yale J Biol Med.* 1965;38:11–36.
15. Furukawa T, Inoue I, Kajiya F, et al. Assessment of biological age by multiple regression analysis. *J Gerontol.* 1975;30:422–434.
16. Webster IW, Logie AR. A relationship between age and health status in female subjects. *J Gerontol.* 1976;31:546–550.
17. Costa PT, McCrae RR. Functional age: a conceptual and empirical critique. In: Haynes SG, Feinleib M, eds. *Epidemiology of Aging*. Washington, US Government Printing Office; 1980:23–46.
18. Costa PT, McCrae RR. Concepts of functional or biological age: a critical view. In: Andres R, Bierman EL, Hazzard WR, eds. *Principle of Geriatric Medicine*. New York: McGraw-Hill; 1985:30–37.
19. Shock NW, Greulich RC, Andres R, et al. *Normal Human Aging: The Baltimore Longitudinal Study of Aging*. Washington, DC: US Government Printing Office; 1984:207–210.
20. Nakamura E. A study on the basic nature of human biological aging processes based upon a hierarchical factor solution of the age-related physiological variables. *Mech Ageing Dev.* 1991;60:153–170.
21. Shock NW. Longitudinal studies of aging in humans. In: Finch CE, Schneider EI, eds. *Handbook of the Biology of Aging*. New York: Van Nostrand Reinhold; 1985:721–743.
22. Rowe JW, Wang SY, Elahi D. Design, conduct, and analysis of human aging research. In: Schneider EL, Rowe JW, eds. *Handbook of the Biology of Aging*, 3rd ed. San Diego, CA: Academic Press; 1990:63–71.
23. Ferris BG. Epidemiology standardization projects. *Am Rev Respir Dis.* 1978;118:55–88.
24. Joreskog KG, Sorom D. *LISREL V: Analysis of Linear Structural Relationships by the Method of Maximum Likelihood.* Chicago, IL: International Educational Services; 1983.
25. Arbuckle JL, Wothke W. *Amos 4.0 User's Guide*. Chicago, IL: Smallwaters Corporation; 1995.
26. Norusis MJ. *SPSS-X Advanced Statistics Guide*. New York: McGraw-Hill; 1985.
27. Ingram DK, Nakamura E, Smucny D, Roth GS, Lane MA. Strategy for identifying biomarkers of aging in long-lived species. *Exp Gerontol.* 2001;36:1025–1034.
28. Guttman L. Some necessary conditions for common factor analysis. *Psychometrika.* 1954;19:149–161.
29. Clark JW. The aging dimension: a factorial analysis of individual differences with age on psychological and physiological measurements. *J Gerontol.* 1960;15:183–187.
30. Jalavisto E, Makkonen T. On the assessment of biological age. II. A factorial study of aging in postmenopausal women. *Ann Acad Sci Fenn Med.* 1963;101:5–35.
31. Nakamura E, Miyao K, Ozeki T. Assessment of biological age by principal component analysis. *Mech Ageing Dev.* 1988;46:1–18.
32. Hochschild R. Can an index of aging be constructed for evaluating treatments to retard aging rates? A 2462-person study. *J Gerontol Biol Sci.* 1990;45:B187–B214.
33. Guilford JP. *Psychometric Methods*, 2nd ed. New York: McGraw-Hill; 1954:470–538.

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