THE GERONTOLOGICAL SOCIETY OF AMERICA®

*Journals of Gerontology: Medical Sciences*
cite as: *J Gerontol A Biol Sci Med Sci*, 2021, Vol. 76, No. 9, 1633–1642
doi:10.1093/gerona/glaa238
Advance Access publication September 18, 2020

OXFORD

Research Article

# Development and Validation of 2 Composite Aging Measures Using Routine Clinical Biomarkers in the Chinese Population: Analyses From 2 Prospective Cohort Studies

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Received: May 6, 2020; Editorial Decision Date: September 7, 2020

**Decision Editor:** Anne B. Newman, MD, MPH, FGSA

## Abstract

**Background:** This study aimed to: (i) develop 2 composite aging measures in the Chinese population using 2 recent advanced algorithms (the Klemera and Doubal method and Mahalanobis distance); and (ii) validate the 2 measures by examining their associations with mortality and disease counts.

**Methods:** Based on data from the China Nutrition and Health Survey (CHNS) 2009 wave ($N$ = 8119, aged 20–79 years, 53.5% women), a nationwide prospective cohort study of the Chinese population, we developed Klemera and Doubal method-biological age (KDM-BA) and physiological dysregulation (PD, derived from Mahalanobis distance) using 12 biomarkers. For the validation analysis, we used Cox proportional hazard regression models (for mortality) and linear, Poisson, and logistic regression models (for disease counts) to examine the associations. We replicated the validation analysis in the China Health and Retirement Longitudinal Study (CHARLS, $N$ = 9304, aged 45–99 years, 53.4% women).

**Results:** Both aging measures were predictive of mortality after accounting for age and gender (KDM-BA, per 1-year, hazard ratio [HR] = 1.14, 95% confidence interval [CI] = 1.08, 1.19; PD, per 1-*SD*, HR = 1.50, 95% CI = 1.33, 1.69). With few exceptions, these mortality predictions were robust across stratifications by age, gender, education, and health behaviors. The 2 aging measures were associated with disease counts both cross-sectionally and longitudinally. These results were generally replicable in CHARLS although 4 biomarkers were not available.

**Conclusions:** We successfully developed and validated 2 composite aging measures—KDM-BA and PD, which have great potentials for applications in early identifications and preventions of aging and aging-related diseases in China.

**Keywords:** Aging measure; Chinese population; Klemera and Doubal method; Mortality; Physiological dysregulation

Aging is one of the leading risk factors for most chronic diseases (1,2). Increased population aging has been a great public health burden to the global society. One key question for addressing aging and related issues is to quantify aging. However, this is challenging as aging is a complex and multifactorial process characterized by degeneration and loss of function across multiple physiological systems. Intuitively, selecting multisystem clinical biomarkers that reflect functioning or state, and applying appropriate algorithms/methods to develop composite aging measures are plausible. Such composite aging measures have been reported in literature (3–16), showing increased use of advanced statistical algorithms/methods. One of the commonly used algorithms/methods was proposed by Klemera and Doubal (17). The biological age (BA) estimated using the Klemera and Doubal method (referred to as KDM-BA) has been favored in terms of its great potential of predicting aging outcomes including mortality and physical dysfunction (3,18). The KDM-BA

has been widely used in aging and aging-related studies (3–5,7–10), despite the complexity in computing and the controversy around the inclusion of chronological age (CA) as a biomarker (5,6). Recently, a statistical distance (ie, Mahalanobis distance) was proposed by Cohen and colleagues to quantify aging by extracting the information about a number of deviations of multisystem clinical biomarkers from a specified "physiological norm" or a baseline state, resulting in a single measure termed as physiological dysregulation (PD) (11). Despite that PD does not directly estimate BA, it captures preclinical manifestation of transition from healthy to unhealthy states (16) and is a promising aging measure in terms of its association with mortality and diseases (11–16).

Due to differences in many factors such as genetic predisposition, demographics, and socioeconomic circumstances, aging measures developed in one population may not be generalizable to another population (19,20). This therefore requires separate efforts in each population and is crucial when facing a population with unique characteristics such as the Chinese population. On the one hand, China is facing unprecedented rapid population aging and has the largest older population (ie, >200 million older adults over 60 years live in China in 2015) (21). On the other hand, the Chinese older population has experienced great demographic (eg, the Great Chinese Famine during 1959–1961), economic (eg, the reform of the economic system in 1978), and sociological changes throughout their life course, which have led to considerable heterogeneity in their aging process. Thus, developing aging measures specific to the Chinese population is of importance and interest to understand numbers of questions on aging in China. To date, a few research groups have reported several composite aging measures such as frailty index (22–24) and BA (25–29) for the Chinese population in Mainland; whereas no studies on KDM-BA and PD, 2 promising aging measures, have been reported. In addition, limited previous studies have explored the question of whether the aging measures work well in population subgroups (eg, those healthy individuals without clinical diagnosed diseases), which is greatly important when it comes to facilitating early identification of individuals at risk (25–29).

In this study, we used data from the China Nutrition and Health Survey (CHNS), a nationwide prospective cohort study of the Chinese population to: (i) develop 2 composite aging measures (ie, KDM-BA and PD) in the Chinese population using 2 most recent advanced algorithms (ie, the Klemera and Doubal method and Mahalanobis distance); (ii) validate the 2 measures by examining their associations with aging outcomes (ie, mortality and disease counts). To strengthen the generalizability of the findings, we replicated the validation analysis in another independent cohort—the China Health and Retirement Longitudinal Study (CHARLS).

## Method

### Study Cohorts—CHNS and CHARLS

The CHNS is an ongoing nationwide prospective cohort study of the Chinese population (covering all age groups), with the major aim of examining across space and time how economic, sociological, and demographic changes affect nutrition and health-related outcomes in the context that China is experiencing rapid transformations in these aspects. The CHNS was initiated in 1989, then repeated in 1991, 1993, 1997, 2000, 2004, 2006, 2009, 2011, and 2015. The details of the sampling design, response rates, attrition, and measures are described elsewhere (30,31). Briefly, the CHNS used a multistage, random-cluster design to recruit participants from 9 provinces (Liaoning, Jiangsu, Shandong, Henan, Hubei, Hunan, Guangxi, Guizhou, and Heilongjiang). By 2011, over 30 000 participants were included in CHNS from the 9 provinces where the total population constituted 47% of China's population (according to the 2010 census) (30,31). In all the waves of the CHNS, information on a wide range of demographic and economic circumstances, diet, behaviors, and health were collected from each household member. Blood samples were collected in 2009 for the first time. Written informed consent was obtained for each participant. The CHNS were approved by the institutional review boards at the University of North Carolina at Chapel Hill and the National Institute of Nutrition and Food Safety.

The CHARLS aims to collect a high-quality nationally representative sample of Chinese community-dwelling individuals aged 45 and older, using a multistage sampling strategy covering 28 provinces, 150 counties/districts, and 450 villages/urban communities across China (32). The participants were first recruited in 2011/2012 (baseline survey) and completed 2 follow-up visits biennially up to 2015. The CHARLS collected a wide range of information on demographic characteristics, socioeconomic status, family structure, chronic disease, physical function, health care and insurance, work, retirement and pension, income and consumption, etc. The CHARLS collected 2 rounds (in 2011/2012 and 2015, respectively) of blood biospecimen. More details of the CHARLS are described elsewhere (32).

First, we included 9535 participants from the 2009 wave of CHNS and who had data on biomarkers (Supplementary Figure S1). Second, to ensure that participants were old enough to be experiencing detectable age-related changes in biomarkers, but not too old as to represent a selected group with above-(or below-) average health (eg, the oldest old ≥ 80 years), we restricted study participants to those aged between 20 and 79 years ($N$ = 8394). Third, due to missing data on biomarkers, additional 275 (3.3%) participants were excluded, leaving a final analytic sample of 8119 participants. In CHARLS, we included participants from the baseline survey because of the availability of blood biomarker and mortality data required for the association analysis. Out of 11 847 participants in the 2011/2012 wave of CHARLS and who provided blood biospecimen, we excluded those with missing data on age ($n$ = 9), aged less than 45 years ($n$ = 270), and with missing data on one or more biomarkers ($n$ = 2264), leaving a final analytic sample of $n$ = 9304 participants (Supplementary Figure S1).

### Biomarker Selection and Development of KDM-BA, KDM-BAacc, and PD

As done in a previous publication (3), we considered candidate biomarkers based on existing knowledge about their role in the aging process, use in previous aging studies, their availability in the data set used, and the statistical significance and strength of their relationships with CA. A total of 25 blood biomarkers were measured in the 2009 wave of CHNS (33), plus systolic and diastolic blood pressure (SBP and DBP), resulting in 27 candidate biomarkers for the first round of considerations in this study. Among them, high correlation ($r$ > 0.7) was observed in a few sets including total cholesterol (TC), low-density lipoprotein cholesterol, and Apolipoprotein B, glycated hemoglobin and glucose, and SBP and DBP. According to Klemera and Doubal (17), we kept one for each set in the subsequent list of biomarkers (TC, glycated hemoglobin, SBP) considering their use in clinical settings and their property. We then selected 12 biomarkers that showed an absolute age

correlation over 0.1. The final list (referred to as the original set) included TC, triglyceride (TG), glycated hemoglobin, urea, creatinine, albumin, high-sensitivity C-reactive protein (hs-CRP), red blood cell count (RBC), platelet count (PLT), ferritin, transferrin, and SBP, representing different domains of physical function: immune function (hs-CRP, RBC, and PLT), cardiac-metabolic function (TC, TG, glycated hemoglobin, ferritin, and SBP), liver function (albumin), and kidney function (urea, creatinine). Before calculating aging measures, non-normally distributed biomarkers (eg, hs-CRP) were log-transformed.

Following procedures described in previous publications (3,17), we calculated KDM-BA for each participant. In brief, the KDM takes information from $m$ number of regression lines of CA regressed on $m$ biomarkers ($m$ = 12 in this study). The final product is KDM-BA in unit of years. To account for the effect of CA, we calculated KDM-BA acceleration (KDM-BAacc), defined as the residual resulting from a linear model when regressing KDM-BA on CA. A score of 0 for KDM-BAacc suggests a KDM-BA that is consistent with what is expected based on one's CA, whereas a positive value suggests that the individual has clinical profile that characterize an older individual, and a negative value suggests the individual has the clinical profile of an individual younger than expected.

For PD, following the procedures described by Cohen and colleagues (11–16), we used the same set of 12 biomarkers above to calculate Mahalanobis distance using a reference population aged 20–39 years. This distance measure was then log-transformed (termed as PD), with a higher value indicating how "strange" each individual's biomarker profile was relative to the reference population mean. Physiological dysregulation was standardized for the subsequent analysis.

Because 4 of the original set of 12 biomarkers (albumin, RBC, ferritin, and transferrin) were not available in CHARLS, we used similar methods mentioned above to calculate KDM-BA, KDM-BAacc, and PD (standardized) based on the remaining 8 biomarkers (referred to as the alternative set of 8 biomarkers).

## Mortality and Disease Counts

In CHNS, the date of death was obtained by the information reported in each wave. We calculated follow-up duration as the time from baseline (survey date in 2009 wave) to death or the censoring time (eg, survey date in 2015) depending on which came first. For those lost to follow-up, we censored them at the middle time of 2009 and 2015 waves (ie, June 2012). Information on the following chronic diseases was collected in 2009, 2011, and 2015 waves: hypertension, diabetes mellitus, myocardial infarction, stroke, hip fracture, asthma, and cancer. We summed them up to obtain a disease counts variable, ranging from 0 to 7. Based on the disease counts, we created a variable with 4 categories—no disease, 1 disease, 2 diseases, and 3 or more diseases.

In CHARLS, the death information was collected from the exit interviews in 2013 and 2015 waves. However, date of death was not provided in 2015 wave. Therefore, we included a binary variable to denote occurrence of death over the 4-year follow-up since baseline (ie, 2011/2012) in this study, instead of calculating the survival time as done in CHNS. A total of 10 self-reported chronic diseases included hypertension, diabetes or high blood sugar, cancer or malignant tumor, chronic lung disease, heart problems, stroke, kidney disease, stomach or other digestive disease, arthritis or rheumatism, and asthma. As done above, we summed them up to obtain a disease count variable (ranging from 0 to 10) and then created a variable with 4 categories—no disease, 1 disease, 2 diseases, and 3 or more diseases. Because no timing for disease incident was provided in both CHNS and CHARLS, in the association analysis when focusing participants who were disease-free at baseline, we included a binary variable to denote occurrence of 1+ disease over the follow-up since baseline, instead of calculating the time-to-event.

## Health and Demographic Characteristics

To account for the confounding effect and conduct further subgroup analyses, we considered the following covariates: age, gender, education, marital status, smoking status, alcohol consumption, and body mass index (BMI), and provided the details in Supplementary Material.

## Statistical Analyses

We presented baseline characteristics (including biomarkers) of the study cohorts using mean (standard deviation [*SD*]) or numbers (percentages). We plotted the distribution of KDM-BAacc and PD, and the correlation between CA and the 2 aging measures (KDM-BA and PD).

As shown in Supplementary Figure S1, we briefly performed 2 main analyses in both CHNS and CHARLS, one for mortality (analysis 1) and another for disease counts (analysis 2). To evaluate the association of the 2 aging measures with all-cause mortality in full sample, we used Cox proportional hazard regression models in CHNS and logistic regression models in CHARLS. Model 1 adjusted for CA and gender. Model 2 additionally adjusted for education, marital status, smoking status, alcohol consumption, and BMI (as categorical variable).

To evaluate whether the 2 aging measures could differentiate mortality risk across various populations, we conducted the mortality analysis in population subgroups by age, gender, education, smoking status, alcohol consumption, disease counts, and BMI category. We also defined a subgroup termed "healthy participants" as those having no disease and normal BMI and repeated the analysis in this subgroup. All models were adjusted for CA and gender, with an exception for gender subgroup analysis (only adjusted for CA).

To evaluate the association of the 2 aging measures with disease counts at baseline in full sample, we first used linear regression models with KDM-BAacc or PD as dependent variables. Based on these regression equations, we estimated the incremental increase in the KDM-BAacc and PD for each of the disease count categories in comparison to participants who were disease-free. Next, we used Poisson regression models to examine the associations of the 2 aging measures with disease counts (disease counts as dependent variables). We ran 2 models: Model 1 adjusted for CA and gender; Model 2 additionally adjusted for education, marital status, smoking status, alcohol consumption, and BMI (as categorical variable). Finally, we focused on the participants who were disease-free at baseline and evaluated associations of KDM-BAacc and PD with disease transition (from no to 1+ diseases) using logistic regression models. Two similar aforementioned models were performed. We did not perform Cox proportional hazard regression models because the timing of developing diseases during follow-up period was unknown.

For the analysis 1 and 2 in full sample above, we presented results in the main text from 3 scenarios as following: (i) using the original set of 12 biomarkers in CHNS; (ii) using the alternative set of 8 biomarkers in CHNS (to evaluate the robustness of the results); and (iii) using the alternative set of 8 biomarkers in CHARLS (a replication analysis).

Additional analyses are detailed in Supplementary Material. We examined the proportional hazard assumption in all Cox proportional hazard regression models above and documented hazard ratios (HRs), corresponding 95% confidence intervals (CIs), and *z*-scores. For Poisson regression models, we documented coefficients (coef.), standard errors (*SE*s), and *z*-scores. For logistic regression models, we documented odds ratios (ORs), corresponding 95% CIs, and *z*-scores. All analyses were performed using SAS 9.4 (SAS Institute, Cary, NC) and R version 3.5.2 (2018-12-20). We considered 2-sided $p$ value < .05 to be statistically significant.

## Results

The basic characteristics of the study cohorts (CHNS and CHARLS) are presented in Table 1. The mean ages of the 8119 participants in CHNS and the 9304 participants in CHARLS were 49.9 (*SD* = 14.1) years and 59.3 (*SD* = 9.4) years, respectively. The proportion of women (53.5% in CHNS and 53.4% in CHARLS) was slightly higher than that of men.

### Characteristics of KDM-BA and PD

In CHNS, KDM-BA ranged from 15.1 to 87.3 years, with a mean and median value of 49.9 and 50.3 years (*SD* = 14.3). Physiological dysregulation ranged from 0.22 to 5.43, with a mean and median value of 2.34 and 2.31 (*SD* = 0.60). In CHARLS, KDM-BA ranged from 32.0 to 95.2 years, with a mean and median value of 57.3 and 56.3 years (*SD* = 10.2). Physiological dysregulation ranged from −0.90 to 4.71, with a mean and median value of 1.89 and 1.87 (*SD* = 0.67). Figure 1 presents the characteristics of KDM-BA, KDM-BAacc, and PD. As expected, KDM-BA and CA were highly correlated (Figure 1E and F), partially because age is in the KDM-BA measure (17). Physiological dysregulation showed significant but weak correlation with CA (Figure 1G and H).

### Associations of KDM-BA and PD With All-Cause Mortality in Full Sample

Table 2 shows the associations of KDM-BA and PD with all-cause mortality in full sample. We found that when using the original set of 12 biomarkers in CHNS, 1-year increase in KDM-BAacc increased the risk of mortality by 14% (HR = 1.14, 95% CI = 1.08, 1.19) and each 1-*SD* increase in PD (after adjusting for CA and gender) increased the risk of mortality by 50% (HR = 1.50, 95% CI = 1.33, 1.69). When using the alternative set of 8 biomarkers in CHNS, the above associations became weaker but remained significant. In CHARLS, after adjusting for CA and gender, both KDM-BAacc and PD were significantly associated with higher odds of death (KDM-BAacc, OR = 1.05, 95% CI = 1.03, 1.07; PD, OR = 1.44, 95% CI = 1.31, 1.60). Further adjustment for other covariates including education, marital status, smoking status, alcohol consumption, and BMI did not change these results substantially (Model 2, Table 2). Furthermore, these results remained when disease counts were additionally controlled for (Supplementary Table S1).

### Associations of KDM-BA and PD With All-Cause Mortality in Population Subgroups

Figure 2 presents associations of KDM-BAacc and PD with all-cause mortality in population subgroups (detailed results can be found in Supplementary Tables S2 and S3). Overall, we found consistent results in nearly all of these subgroups regardless of

**Table 1.** Characteristics of the Study Cohorts

| | CHNS ($n$ = 8119) | CHARLS ($n$ = 9304) |
|---|---|---|
| Age, y | 49.9 ± 14.1 | 59.3 ± 9.4 |
| Young and middle-aged adults (<60 y) | 5978 (73.6) | 5115 (55.0) |
| Older adults (≥60 y) | 2141 (26.4) | 4189 (45.0) |
| Gender | | |
| Women | 4342 (53.5) | 4972 (53.4) |
| Men | 3777 (46.5) | 4332 (46.7) |
| Education | | |
| No schooling | 1844 (25.8) | 2802 (30.1) |
| Primary school | 1624 (22.7) | 3823 (41.1) |
| Middle school | 2717 (38.0) | 1804 (19.4) |
| High school or more | 971 (13.6) | 875 (9.4) |
| Marital status | | |
| Currently married | 6990 (86.5) | 7763 (83.4) |
| Others | 1096 (13.6) | 1541 (16.6) |
| Smoking status | | |
| Nonsmoker | 5849 (72.1) | 5641 (60.9) |
| Smoker | 2266 (27.9) | 3622 (39.1) |
| Alcohol consumption | | |
| Nondrinker | 5445 (67.1) | 5673 (61.1) |
| Drinker | 2671 (32.9) | 3615 (38.9) |
| BMI categories* | | |
| Underweight | 482 (6.0) | 629 (6.9) |
| Normal | 4281 (53.2) | 4793 (52.3) |
| Overweight | 2482 (30.8) | 2681 (29.2) |
| Obese | 805 (10.0) | 1065 (11.6) |
| Disease counts | | |
| 0 | 6544 (80.6) | 2778 (29.9) |
| 1 | 1245 (15.3) | 2964 (31.9) |
| 2 | 281 (3.5) | 2035 (21.9) |
| 3+ | 47 (0.6) | 1519 (16.3) |
| Healthy† | 3650 (45.3) | 1607 (17.3) |
| Died | 221 (2.7) | 394 (4.2) |
| Biomarkers selected for calculating KDM-BA and PD | | |
| Total cholesterol, mmol/L | 4.9 ± 1.0 | 5.0 ± 1.0 |
| Log(Triglyceride), mmol/L | 0.3 ± 0.6 | 0.2 ± 0.5 |
| Glycated hemoglobin, % | 5.6 ± 0.9 | 5.3 ± 0.8 |
| Urea, mmol/L | 5.5 ± 1.5 | 5.6 ± 1.6 |
| Creatinine, μmol/L | 86.9 ± 17.1 | 68.9 ± 16.5 |
| Albumin, g/L | 47.5 ± 3.4 | — |
| Log(high-sensitivity C-reactive protein), mg/L | −0.07 ± 1.5 | 0.2 ± 1.1 |
| Red blood cell count, $10^{12}$ cells/L | 4.7 ± 0.7 | — |
| Platelet count, $10^9$ cells/L | 213.2 ± 67.3 | 212.0 ± 72.8 |
| Log(Ferritin), ng/mL | 4.3 ± 1.2 | — |
| Transferrin, mg/dL | 288.3 ± 55.6 | — |
| Systolic blood pressure, mmHg | 124.5 ± 18.8 | 130.9 ± 21.6 |

*Notes*: BMI = body mass index; CHARLS = China Health and Retirement Longitudinal Study; CHNS = China Health and Nutrition Survey; KDM-BA = Klemera and Doubal method-biological age; PD = physiological dysregulation; *SD* = standard deviation. Values are presented as mean ± *SD* or No. (%). Percentages may not sum to 100 because of rounding. There were missing data on ethnicity ($n$ = 27), education ($n$ = 963), marital status ($n$ = 33), smoking status ($n$ = 4), alcohol consumption ($n$ = 3), BMI categories ($n$ = 69), and disease ($n$ = 2) in CHNS. There were missing data on smoking status ($n$ = 41), alcohol consumption ($n$ = 16), BMI categories ($n$ = 136), and disease ($n$ = 8) in CHARLS.

*BMI was calculated as weight in kilograms divided by height in meters squared. Underweight was defined as BMI < 18.5 kg/m$^2$; normal was defined as 18.5 ≤ BMI < 24.0 kg/m$^2$; overweight was defined as 24.0 ≤ BMI < 28.0 kg/m$^2$; and obese was defined as BMI ≥ 28 kg/m$^2$.

†Healthy participants were defined as those having no disease and normal BMI.

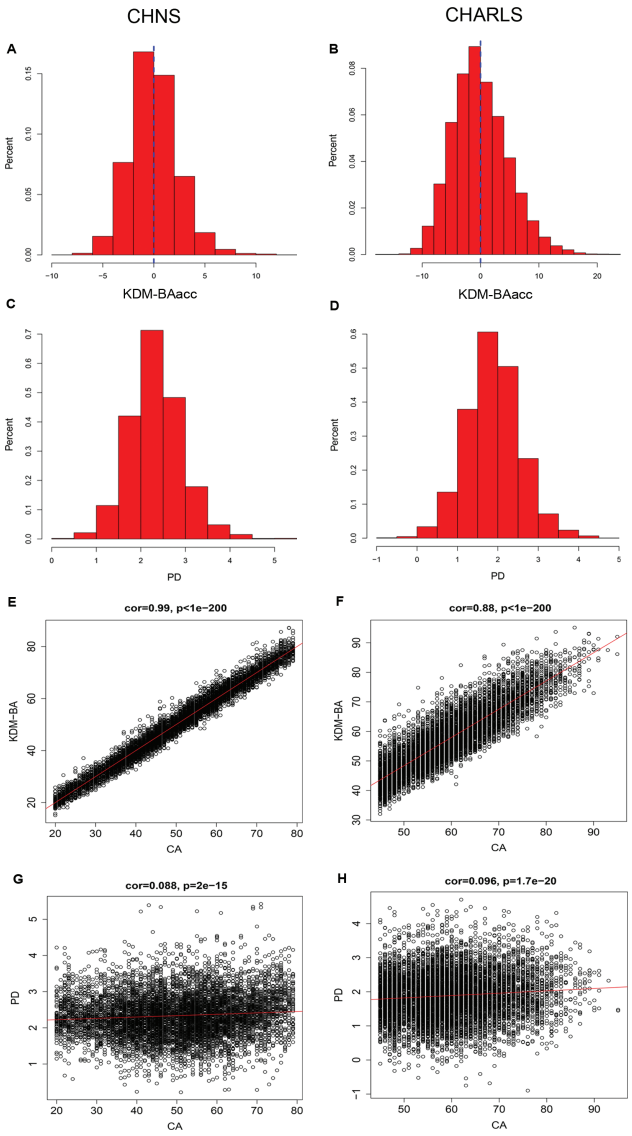


**Figure 1.** Characteristics of KDM-BA, KDM-BAacc, and PD. KDM-BA = Klemera and Doubal method-biological age; KDM-BAacc = Klemera and Doubal method-biological age acceleration; PD = physiological dysregulation; CA = chronological age. **A** and **B**, and **C** and **D** show the distribution of KDM-BAacc and PD, respectively. **E** and **F**, and **G** and **H** show the correlation between CA and the 2 measures (KDM-BA and PD), respectively. **A**, **C**, **E**, and **G** are based on the China Health and Nutrition Survey (CHNS). **B**, **D**, **F**, and **H** are based on the China Health and Retirement Longitudinal Study (CHARLS). Full color version is available within the online issue.

study cohorts (CHNS and CHARLS) and aging measures (KDM-BAacc and PD). For example, when stratified by age, gender, education, smoking status, or alcohol consumption in CHNS, the HR of KDM-BAacc for mortality ranged from 1.11 (older adults) to 1.27 (high school or more), consistent with that in the full sample (HR = 1.14, Table 2). Similar results were found for PD, such that HR ranged from 1.36 (women) to 1.77 (men) in comparison to that in the full sample (HR = 1.50). In participants who were disease-free or who had normal BMI, we found that both KDM-BAacc and PD were associated with mortality, with the exception that a high HR of 1.05 was found in those who were disease-free in CHNS. In those who were defined as healthy (ie, having no disease and normal BMI), we found that both KDM-BAacc and PD were associated with mortality (eg, in CHNS, KDM-BAacc, HR = 1.18, 95% CI = 1.05, 1.31; PD, HR = 1.65, 95% CI = 1.33, 2.05).

### Associations of KDM-BA and PD With Disease Counts in CHNS

Figure 3 shows predicted increases in KDM-BAacc and PD for each disease count, compared to participants who were disease-free. Overall, participants with disease had higher KDM-BAacc and PD compared to those without disease. Such that in CHNS, those with 1 disease were 1.18 years older, those with 2 diseases were about 1.89 years older, and those with 3 and more diseases were 2.44 years older than those without disease. The PD for those with 1 disease was higher by 0.22, for those with 2 diseases was higher by 0.54, and for those with 3 and more diseases was higher by 0.79, relative to that for those without disease. This pattern was observed when using the alternative set of 8 biomarkers in CHNS (Figure 3C and D) and in CHARLS (Figure 3E and F), although the absolute values were slightly different.

To gain further insights into the relations between the 2 aging measures and disease counts, we used Poisson regression models to examine the association of KDM-BAacc and PD with disease counts in full sample (Supplementary Table S4). After accounting for CA and gender, both KDM-BAacc and PD were significantly associated with disease counts (KDM-BAacc, coef. = 0.19, *SE* = 0.008; PD, coef. = 0.21, *SE* = 0.021). Similar results were observed when using the alternative set of 8 biomarkers in CHNS and in CHARLS. The results maintained even after accounting for more covariates including education, marital status, smoking status, alcohol consumption, and BMI.

For those who were disease-free at baseline, we further examined the association of KDM-BAacc and PD with disease transition (Supplementary Table S5). Overall, higher KDM-BAacc and PD were associated with higher odds of developing 1+ diseases in both CHNS and CHARLS, although the association of PD with disease transition was marginally significant in CHARLS. We further adjusted for more covariates and found similar results (Model 2, Supplementary Table S5).

## Discussion

In this study, we developed and validated 2 composite aging measures—KDM-BA and PD in the Chinese population. We demonstrated that the 2 aging measures were highly predictive of mortality, accounting for CA and gender. With few exceptions, these mortality predictions were robust across different stratifications, particularly by age, gender, education, and health behaviors. Furthermore, the 2 aging measures were associated with disease counts both cross-sectionally and longitudinally. These disease associations did not necessarily have influence on the mortality predictions by the 2 aging measures, indicating that the 2 aging measures did not just capture diseases but might track the effect of aging before diseases become clinically evident. The above results were replicable in another independent cohort—CHARLS, despite fewer biomarkers used. Overall, we provide 2 promising aging measures for the Chinese population, which can be used to explore numerous questions relevant to aging (eg, serving as proxies of life span in geroprotective therapies (34)) in China, the largest developing country with rapidly growing aging population.

**Table 2.** Associations of KDM-BAacc and PD With All-Cause Mortality in Full Sample

| | | Model 1 | | | Model 2 | | |
|---|---|---|---|---|---|---|---|
| | | HR/OR (95% CI) | *z*-Score | *p* Value | HR/OR (95% CI) | *z*-Score | *p* Value |
| 12 biomarkers in CHNS | | | | | | | |
| KDM-BAacc | Per year | 1.14 (1.08, 1.19) | 4.99 | <.001 | 1.16 (1.10, 1.22) | 5.42 | <.001 |
| PD | Per *SD* | 1.50 (1.33, 1.69) | 6.64 | <.001 | 1.49 (1.31, 1.68) | 6.15 | <.001 |
| 8 biomarkers in CHNS | | | | | | | |
| KDM-BAacc | Per year | 1.05 (1.03, 1.08) | 3.99 | <.001 | 1.06 (1.03, 1.09) | 4.47 | <.001 |
| PD | Per *SD* | 1.45 (1.29, 1.64) | 6.03 | <.001 | 1.43 (1.25, 1.62) | 5.44 | <.001 |
| 8 biomarkers in CHARLS | | | | | | | |
| KDM-BAacc | Per year | 1.05 (1.03, 1.07) | 4.44 | <.001 | 1.06 (1.03, 1.08) | 5.03 | <.001 |
| PD | Per *SD* | 1.44 (1.31, 1.60) | 7.20 | <.001 | 1.47 (1.32, 1.63) | 7.20 | <.001 |

*Notes*: CHARLS = China Health and Retirement Longitudinal Study; CHNS = China Health and Nutrition Survey; CI = confidence interval; HR = hazard ratio; KDM-BAacc = Klemera and Doubal method-biological age acceleration; OR = odds ratio; PD = physiological dysregulation; *SD* = standard deviation. As described in Method section, date of death was available in CHNS. Thus, Cox proportional hazard regression methods were used and HRs (95% CIs) were documented in CHNS. Since date of death was not provided in 2015 wave of CHARLS, we included a binary variable to denote occurrence of death over the 4-year follow-up since baseline in this study, rather than calculating the survival time as done in CHNS. Therefore, we used logistic regression models to examine the associations of KDM-BA and PD with death and documented ORs (95% CIs) in CHARLS. Model 1 adjusted for age and gender. Model 2 additionally adjusted for education, marital status, smoking status, alcohol consumption, and body mass index (BMI) (as categorical variable). The sample size was 8177 in the analysis when using "8 biomarkers in CHNS."

To the best of our knowledge, this is the first study to apply 2 most recent advanced algorithms (ie, the Klemera and Doubal method and Mahalanobis distance) to develop composite aging measures in Mainland China. More importantly, the development was based on a large number of Chinese adults in a national wide study design and was further validated by linking the aging measures to important health outcomes: mortality and disease counts, which were not covered in previous studies in Mainland China (25–29). The efficacy of the 2 aging measures to assess risk of mortality and disease counts provides strong evidence of their suitability for potential applications in both large-scale epidemiological studies and clinical settings, as well as basic research on biological aging. For example, one may apply the 2 aging measures to evaluate the roles of large numbers of factors (eg, green tea drinking and playing mahjong, which are popular in China) in the healthy aging in the Chinese population. Moreover, one can think of these aging measures as surrogate markers of life span to evaluate the effectiveness of antiaging interventions and therapies in the Chinese population, which would save considerable time for subsequent long-term follow-up (34–38).

The robust mortality predictions of the 2 aging measures across different population subgroups further strengthen their public implications. Particularly, both KDM-BA and PD predicted mortality in the healthy subgroup in this study, indicating that they may capture preclinical manifestations of many diseases or other factors underlying the aging process. This is similar to the development of a frailty index based on laboratory test results (FI-Lab) (39), because the subclinical deficit accumulation reflected by FI-Lab increased risks of adverse outcomes (eg, mortality) even after accounting for the conventional frailty index (40–44). In addition, in those who were disease-free at baseline, we observed the strong association between accelerated aging and disease development, which further implies that the aging measures may capture certain physiopathological processes preceding diseases, supporting the Geroscience paradigm (1,2). These findings emphasize the potential applications of the 2 aging measures in basic research on aging and in younger populations who appear healthy. These applications such as early preventions should be highly cost-effective.

Although direct comparisons on KDM-BA and PD and their predictive power of mortality and disease across different countries/regions and populations are somewhat problematic due to many differences in participants characteristics, biomarkers selection, and follow-up time, etc., it is important to place our findings in the international context. To date, the majority of investigations on KDM-BA and PD have been conducted in the United States (particularly focusing on Whites and African American) (3,7,8,11,16), and the remaining in Canada (the Canadian Study of Health and Aging) (5), Italy (the Invecchiare in Chianti study) (12), Denmark (the Long Life Family Study) (45), New Zealand (the Dunedin Study) (4), Singapore (the Singapore Longitudinal Aging Study) (9), Korea (the Korea National Health and Nutrition Examination Survey) (18), and Taiwan (the Social Environment and Biomarkers of Aging Study) (10). Overall, these investigations reported consistent associations of KDM-BA and/or PD with mortality, despite with slightly different strengths, supporting the applications of the 2 aging measures across countries/regions and populations. Interestingly, the association of KDM-BA with mortality in this study is comparable to that from the first and only study of KDM-BA on U.S. general population and covering a wide range age groups (3). The association of PD with mortality in this study seems to be stronger than that in literature (mainly in United States, eg, (16)), partly due to the wide age ranges of study populations we included. The findings across different studies confirm that KDM-BA and PD serve as 2 generalized aging measures (12). But this does not rule out that small differences across different countries/regions and populations exist in terms of the effect of numerous factors, such as genetics, demographics, economics, and lifestyles, on aging (46). For instance, in a population with robustness characteristic (eg, Tsimane (47)) that is not captured by the 2 aging measures, it is possible to observe a relatively weaker association (8). We therefore call for more studies in various countries/regions and populations and head-to-head comparisons to facilitate the understanding of aging measures.

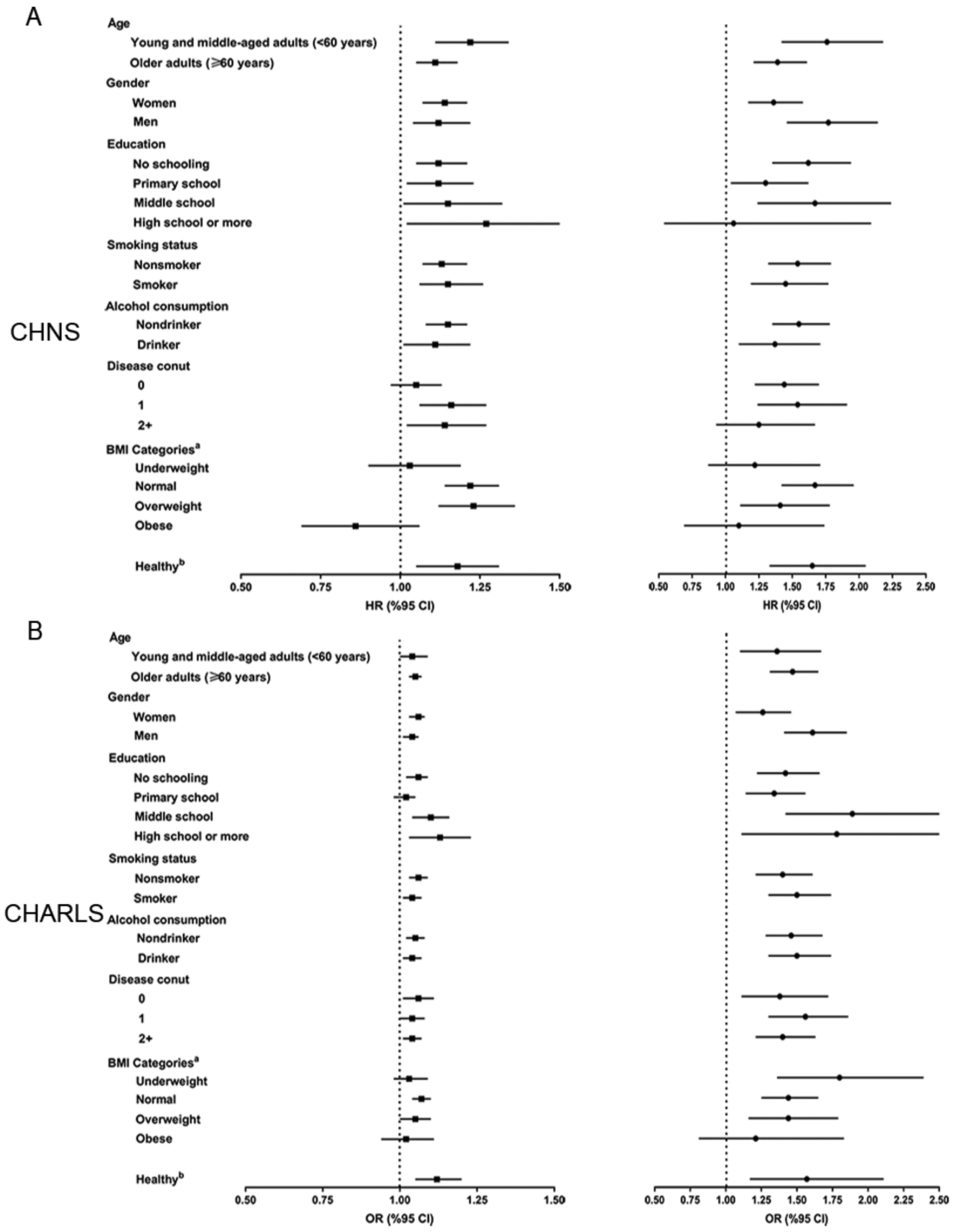


**Figure 2.** Associations of KDM-BAacc and PD with all-cause mortality in population subgroups. KDM-BAacc = Klemera and Doubal method-biological age acceleration; PD = physiological dysregulation (standardized); BMI = body mass index; HR = hazard ratio; OR = odds ratio; CI = confidence interval. **A** and **B** show results from China Health and Nutrition Survey (CHNS) and China Health and Retirement Longitudinal Study (CHARLS), respectively. The left panel in **A** and **B** shows results for KDM-BAacc and the right panel shows those for PD. All models were adjusted for chronological age and gender with an exception for gender subgroup analysis (only adjusted for chronological age). Participants with 2 diseases, and those with 3 or more diseases were combined as one subgroup due to the small sample size in each group. Body mass index was calculated as weight in kilograms divided by height in meters squared. Underweight was defined as BMI < 18.5 kg/m$^2$; normal was defined as 18.5 ≤ BMI < 24.0 kg/m$^2$; overweight was defined as 24.0 ≤ BMI < 28.0 kg/m$^2$; and obese was defined as BMI ≥ 28 kg/m$^2$. Healthy participants were defined as those having no disease and normal BMI.

In this study, we noticed slight differences between KDM-BA and PD although we did not aim to compare them, considering their similarities reported in literature (Table 2, Supplementary Figure S2 and Supplementary Table S4) (48,49). It seems that PD had stronger mortality predictive utility while KDM-BA had stronger disease predictive utility. The slight difference between KDM-BA and PD is largely due to the difference in algorithms/methods that they were derived from, leading to their unique characteristics. For

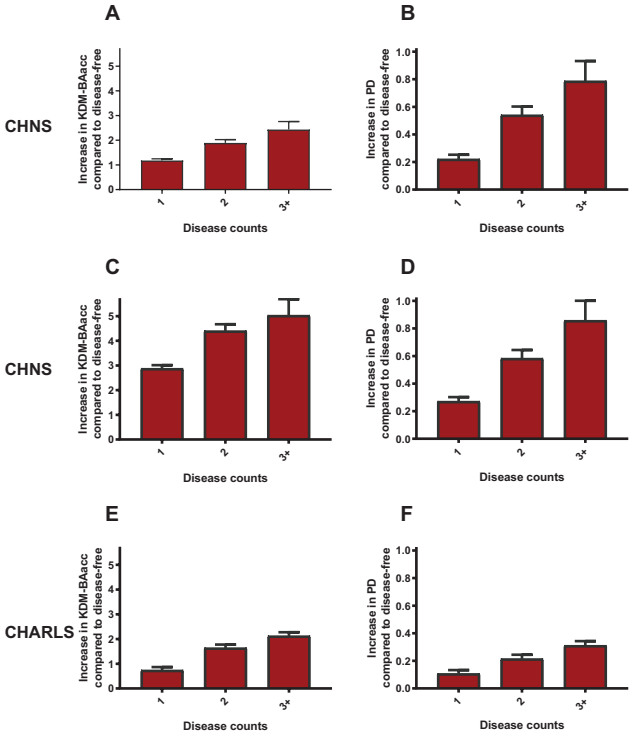


**Figure 3.** Predicted increases in the KDM-BAacc (**A**, **C**, and **E**) and PD (**B**, **D**, and **F**) for each disease count. KDM-BAacc = Klemera and Doubal method-biological age acceleration; PD = physiological dysregulation (standardized); CHNS = China Health and Nutrition Survey; CHARLS = China Health and Retirement Longitudinal Study. The y-axis depicts the increase in KDM-BAacc or PD (standardized) in comparison to participants who were disease-free. The x-axis shows groups categorized based on disease counts that each participant had. The bar indicates standard error. The results are based on a series of linear regression models with adjustment for gender. **A** and **B** show results from CHNS using the original set of 12 biomarkers. **C** and **D** show results from CHNS using the alternative set of 8 biomarkers. **E** and **F** show results from CHARLS using the same 8 biomarkers. Full color version is available within the online issue.

example, KDM-BA is a BA estimate including CA, and has some computing complexities; whereas PD is on the opposite. As mentioned in previous studies in U.S. population (7,48,49), it is probable that clinical biomarker-based composite aging measures are complementary, which needs further specific demonstrations.

The major strengths of this study included the large sample size and the nationwide prospective cohort study nature, which enabled us to develop and validate such aging measures in the study population. In addition, CHARLS provided us a unique opportunity to further validate the aging measures in an independent manner. Meanwhile, several limitations of this study should be noted. First, the CHNS has relatively short follow-up period (ie, up to 6 years), making us unable to examine the long-term effect of aging measures on the outcomes. Second, we were unable to conduct a cause-specific mortality analysis due to the unavailability of data. Third, we did not have data on timing of the diseases we included. Finally, 4 biomarkers were not available in CHARLS, limiting the validation conducted; however, CHARLS is the only independent cohort that has information on these biomarkers and relatively large sample size currently in China.

In summary, we developed 2 multisystem clinical biomarkers-based aging measures—KDM-BA and PD in the Chinese population, which were demonstrated to be associated with mortality and disease counts. The mortality predictions by them are robust to population characteristics. When facing increasing disease burden resulted from increased population aging, focusing on aging—the leading risk factor of diseases would be cost-effective. Thereby, the affordability and practicality of the 2 aging measures we developed have great potentials for applications, particularly for early identifications and preventions of aging and aging-related diseases in China.

## Supplementary Material

Supplementary data are available at *The Journals of Gerontology, Series A: Biological Sciences and Medical Sciences* online.

## Funding

The CHNS was supported by the National Institute for Nutrition and Health, China Center for Disease Control and Prevention, Carolina Population Center (P2C HD050924, T32 HD007168), the University of North Carolina at Chapel Hill, the NIH (R01-HD30880, DK056350, R24 HD050924, and R01-HD38700), and the National Institutes of Health Fogarty International Center (D43 TW009077, D43 TW007709) for financial support for the CHNS data collection and analysis files from 1989 to 2015 and future surveys, and the China-Japan Friendship Hospital, Ministry of Health for support for CHNS 2009, Chinese National Human Genome Center at Shanghai since 2009, and Beijing Municipal Center for Disease Prevention and Control since 2011. We thank the National Institute on Aging (NIA) in the United States (1-R21-AG031372-01, 1-R21-AG033675-01-A1, 1-R01-AG037031-01, and 1-R01-AG037031-03S1), the National Natural Science Foundation of China (70773002, 70910107022, and 71130002), and the World Bank (7159234) for the support for the CHARLS. The current study was conducted at the School of Public Health and the Second Affiliated Hospital, Zhejiang University School of Medicine (Hundred Talents Program). This study was supported by the Fundamental Research Funds for the Central Universities. The funders had no role in the study design; data collection, analysis, or interpretation; in the writing of the report; or in the decision to submit the article for publication.

## Acknowledgments

We appreciate all participants who attended the China Health and Nutrition Survey (CHNS) and China Health and Retirement Longitudinal Study (CHARLS). We thank Dr. Morgan Levine for the assistance in the data analysis and results interpretation. We thank Dr. George Agogo for comments and editing assistance.

## Conflict of Interest

None declared.

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