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Journal:	*BMJ Open*
Manuscript ID	bmjopen-2020-045369
Article Type:	Original research
Date Submitted by the Author:	01-Oct-2020
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Keywords:	MENTAL HEALTH, PUBLIC HEALTH, EPIDEMIOLOGY

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The Association between Allostatic Load and Mortality among Chinese Older Adults: The Chinese Longitudinal Health and Longevity Study

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Word count: 3526

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Abstract

Background: Allostatic load has shown that high burden of AL is associated with increased risk of adverse outcomes, but little attention has been paid to China with largest aging population in the world.

Objective: This study is to examine the association between allostatic load (AL) and all-cause mortality among Chinese adults aged at least 60 years.

Design: Population-based prospective cohort study.

Setting: In 2011-2012, an ancillary study, in which a blood test was added, including a total of 2,439 participants, was conducted in eight longevity areas in the Chinese Longitudinal Healthy Longevity Survey.

Participants: The final analytic sample consisted of 1,519 participants (mean ± SD age: men 80.5±11.3 years; women 90.2±11.8 years; and 53% women).

Primary outcome measure: Cox models were used to examine the association between AL and mortality among men and women, separately. Analysis were also adjusted for potential confounders including age, ethnicity, education, and marital status, smoking and exercise.

Results: Male with a medium AL burden (score: 2-4) and high AL burden (score: 5-9) had a 33% and 118% higher hazard of death, respectively, than those with a low AL burden (score: 0-1). We did not find significant difference between females with different levels of AL burden.

Conclusion: Higher AL burden was associated with increased all-cause mortality among Chinese men aged at least 60 years. However, we did not find strong association among women. In conclusion, Intervention programs targeting modifiable components of the AL burden may help prolong lifespan for older adults, especially men, in China.

Keywords: Allostatic load; Mortality; China; Older adults.

ARTICLE SUMMARY

Article focus

- Is a higher burden of AL associated with increased risk of all-cause mortality among both older men and women in China?

Key messages

- Higher AL burden was associated with increased all-cause mortality among Chinese men aged at least 60 years.
- We did not find strong evidence about Allostatic load was associated with specific causes of death over the same follow-up period among women.

Strengths and limitations of this study

- This is the first to investigate the association between AL and mortality using a Chinese population.
- The CLHLS dataset that is a large nationally representative old population survey in China.
- The updated quartile risk method for biomarkers BMI, total cholesterol, and triglyceride

among older adults.

- We did not include any primary neuroendocrine biomarkers such as cortisol in constructing the AL score.
- There is huge loss to follow up (>20%; 552 of 2439), although only 3 to 7-year range of follow up (2011 to 2014-18), which may underestimate the association.

The Association between Allostatic Load and Mortality among Chinese Older Adults: The Chinese Longitudinal Health and Longevity Study

BACKGROUND

Allostatic load (AL) is conceptualized as the cumulative wear and tear on multiple physiological systems resulting from repeated adaptation to stressors [17,23,24]. In the absence of a gold standard, many operational definitions of AL have been proposed. The most commonly used construct of AL was developed by Seeman and colleagues who have used two categories of biomarkers for quantifying AL [15,24]. The first category (called primary mediators) includes biomarkers the body releases in response to stress, such as cortisol and dehydroepiandrosterone sulphate (DHEA-S); the second category comprises comprises secondary outcomes that result from the effects of primary mediators. The examples of biomarkers are blood pressure (BP), cholesterol, and waist-hip ratio [24].

A number of studies identified that a high burden of AL is associated with increased risk of adverse outcomes including cardiovascular disease, functional decline, and mortality among older adults [1,13,14,17,18,20,25]. For example, in a 7-year longitudinal study conducted in 2006, increased AL score was associated with higher mortality among older population [18]. In a cohort study of 1,023 community-dwelling older adults in Taiwan, researchers found that higher AL score was related with higher death rate [14]. Additionally, some studies found that women and men experienced chronic stress in different ways. For example, Yang et al in 2011 revealed gender difference in the AL biomarkers and the age trajectories of physiological dysregulation [33]. Women had higher level of inflammation biomarkers but lower risk of cardiovascular disease (CVD) than men. Another study from Tampubolon and Maharani in 2018 among older population found that AL score increased in sex difference [34]. Compared

with men, women showed advantage in life expectancy [34]. Taken together, these results suggest the use of sex-specific cut-off points for AL biomarkers in future research.

A number of studies have examined the association between AL and mortality among older adults. However, little attention has been paid to less developed regions, including China – the most populous country with the largest aging population in the world. In 2019, there were 249 million adults aged 60 years or above in China, accounting for 17.3% of its total population, and this number is projected to almost double in 2050, reaching 487 million [4,5]. Understanding the relationship between AL and mortality in less developed country is beneficial for leading to interventions, which could be helpful to change unhealthy lifestyle, decrease morbidity and mortality among older population. In addition, less studies focus on sex-specific cut off points for calculating AL index, this study will conduct sex-specific studies, which may closely reflect AL score among older population.

In this study, we used a large cohort study to examine the association between AL and all-causes mortality among Chinese men and women aged at least 60 years. We hypothesized that a higher burden of AL would be associated with increased risk of all-cause mortality among both older men and women in China.

METHODS

Data and Study Participants

We used data from the Chinese Longitudinal Healthy Longevity Survey (CLHLS), an ongoing prospective, longitudinal study with the largest sample of the oldest old in China. Half of the counties and cities in 22 of the 31 provinces in China (covering 85% of the population) were randomly selected through a multistage cluster sampling approach. A wide range of socio-

demographic, lifestyle, and health measures were collected in the CLHLS. The baseline survey was conducted in 1998 and participants who were alive were re-interviewed in each follow-up survey (2000, 2002, 2005, 2008-2009, 2011-2012, 2014, and 2017-2018). In 2011-2012, an ancillary study, in which a blood test was added, was conducted in eight longevity areas: Laizhou City in Shandong Province, Xiayi County in Henan Province, Zhongxiang City in Hubei Province, Mayang County in Hunan Province, Yongfu County in Guangxi Autonomous Area, Sanshui District in Guangdong Province, Chengmai County in Hainan Province, and Rudong County in Jiangsu Province. The Research Ethics Committees of Peking University and Duke University granted approval for the Protection of Human Subjects for the CLHLS. All study participants gave informed consent. A more detailed description of the recruitment strategy and study design of the CLHLS has been published elsewhere [11,31,32]. A total of 8,959 individuals were included at baseline (1998). 1998 baseline survey, which was extended to 11,162 in 2000, it was found that almost 30 percent died before 2002 interview and approximately 14 percent were lost that was higher than the attrition rate between 1998 and 2000 wave (9.6 percent); the number of participants were extend to 16,064 in 2002, and about 13.8 percent were lost between 2002 and 2005; the number of interviewed were 15,638 in 2005, and about 13.2 percent were lost between 2005 and 2008-2009; the number of participants were extended to 16.540, and approximately 17.7 percent were lost between 2008-2009 and 2011-2012; the total number of interviewed participants were 9765 in 2011-2012 [35].

A total of 2,439 persons contributed blood sample in the ancillary study (2011-2012). Participants were excluded from the analytic sample if they had (i) incomplete data on any biomarkers for constructing AL (n = 251), (ii) no follow-up data (time to death or censorship was undetermined; n = 552), (iii) had extreme values on the biomarkers (n = 109), or (iv) were less than 60 years old (n=16). The final analytic sample consisted of 1,519 participants. We did

not observe appreciable differences in age, ethnicity, marital status, smoking, or chronic conditions between the analytic sample and those excluded (n = 920; Table S1). Compared to the analytic sample, excluded persons had higher education level and higher prevalence of exercise. In addition, compared to people who were loss follow up, the included people had higher prevalence of married, and higher prevalence of stroke; we did not observe appreciable differences in age, ethnicity, smoking, exercise, hypertension, diabetes, heart disease, pulmonary, arthritis, and cancer between both (Table S4).

Calculation of AL Score

Based on previous research [2,9,14,23] and availability of data in the CLHLS, we selected nine biomarkers to construct AL: heart rate, systolic BP (SBP), and diastolic BP (DBP), body mass index (BMI), total cholesterol, high density lipoprotein (HDL) cholesterol, glucose, triglyceride, and C-reactive protein (CRP). BMI, heart rate, SBP, and DBP were collected from physical examinations. BMI was calculated as body weight (kilograms) divided by height (meters) squared. SBP and DBP were measured by a mercury sphygmomanometer with an appropriately sized cuff, taken in the seated position after 5 minutes of quiet rest under the supervision of trained research assistants. We used the average of two measurements for further analyses. Blood samples were used for assays of the level of the total cholesterol, HDL cholesterol, glucose, triglyceride, and CRP.

To be in line with previous studies [6,8,24,25], we used the highest quartile for heart rate, SBP, DBP, glucose, and CRP and the lowest quartile for HDL cholesterol to define high-risk group (coded 1). Because BMI, total cholesterol, and triglyceride were inversely associated with mortality among older adults, especially the oldest old [19,29,30], we used the lowest quartile to define high-risk group for these three biomarkers. For participants who self-reported having

been diagnosed with hypertension and heart disease, we classified their SBP, DBP, and glucose into the high-risk category. Similarly, we classified participants' glucose into the high-risk group if they self-reported having been diagnosed with diabetes. The identification of risk quartiles of biomarkers is commonly used to construct AL index [15, 36]. The cut-points of all nine AL components by men and women were presented in Table 1. We constructed the AL score based on the count of biomarkers falling in the high-risk group, ranging from 0 (lowest) to 9 (highest). To be in line with previous studies [37], we then considered using similar cut-off points, classifying the AL score into three categories based on sample distribution: 0-1 (low burden), 2-4 (medium burden), and 5-9 (high burden).

Mortality

The outcome was all-cause mortality. Vital status and date of death (for persons who died by the end of the study) was ascertained by the close family member or village doctor of the deceased participant during the follow-up survey in 2014 and 2017-2018. We calculated the survival time from the date of the baseline interview to the date of last interview (censored) or the death date.

Covariates

Demographic and lifestyle characteristics were collected by interview, including age, sex, ethnicity, education, and marital status, smoking status, and physical exercise. We divided ethnicity into Han and others (minority groups). Years of education were dichotomized as any (one year or more) and no education, which is commonly way used in CLHLS study [38, 39]. Marital status was dichotomized as married and others (widowed, not married, and divorced). Cigarette smoking was categorized as current, past, and non-smoker. Information of exercise was collected using the question "Do you do exercise at present?" and dichotomized into yes

or no. Chronic conditions were measured based on self-reported physician's diagnosis, including hypertension, diabetes, heart disease, stroke, pulmonary disease (including bronchitis, emphysema, pneumonia and asthma), arthritis, and cancer.

Statistical Analyses

All analyses were conducted separately for males and females. We first presented the relative frequency of the AL score using histograms and calculated mean AL score. Then, we described the baseline characteristics of study sample by AL burden (low, medium, and high) using means and SDs for continuous variables and counts and percentages for categorical variables. Characteristics were compared across the three AL categories using analyses of variance for continuous variables and chi-square tests for categorical variables.

We calculated the death rates across three AL categories (low, medium, and high burden). We used the Cox proportional hazards model to determine the unadjusted and adjusted associations between the AL and all-cause mortality. Age, sex, education, and marital status were included in the demographically adjusted models; smoking status, physical exercise, and chronic status including pulmonary disease and arthritis were added in the fully adjusted models. We modelled AL both continuously and in categories.

Furthermore, one sensitivity analysis was undertaken, which aimed to exam if the model results were influenced when we did not use self-reported hypertension or diabetes to classify participants' risk category for BP or glucose.

All tests were two-sided with a significance level of P-value less than 0.05. We conducted all analyses using STATA version 16.0 (Stata Corp, College Station, TX).

Patient and public involvement

This research was done without patient involvement. Patients were not invited to comment on the study design and were not consulted to develop patient-relevant outcomes or interpret the results. Patients were not invited to contribute to the writing or editing of this document for readability or accuracy.

RESULTS

Distribution of Allostatic Load Categories

The distribution of the AL score (range: 0-9) is right-skewed for both males and females; only 13 (1.8%) males and 10 (1.2%) females had a score of 6-9, respectively (Figure 1). The mean AL score was 2.56 (SD=1.47) for males and 2.28 (SD=1.34) for females. For males, 25.5%, 65.3%, and 10.3% had an AL score of 0-1, 2-4, and 5-9, respectively. For females, 28.5%, 65.9%, and 5.6% had an AL score of 0-1 (low burden), 2-4 (medium burden), and 5-9 (high burden), respectively.

Demographic Characteristics

A total of 709 (46.7%) males were included. The average age for males with an AL score of 0-1 (low burden), 2-4 (medium burden), 5-9 (high burden) was 77.6, 81.0, and 84.1 years, respectively (P = 0.042). In addition, we observed significant differences in the prevalence hypertension and diabetes by different AL burden among males.

The study sample included 810 (53.3%) females. The average age for females with an AL score of 0-1 (low burden), 2-4 (medium burden), 5-9 (high burden) was 87.0, 91.3, and 93.6 years, respectively (P < 0.05; Table 2). Compared with men, women were older, less educated, had a lower prevalence of smoking, and were less physically active. Females with a lower AL were

more likely to be married and have any education than woman with higher AL score; they also had a lower prevalence of hypertension, diabetes, and heart disease. We did not observe significant difference in ethnicity, smoking, physical exercise, stroke, pulmonary disease, arthritis, and cancer across AL burden (low, medium, and high) among females.

Association between Allostatic Load and Mortality among Males

A total of 310 males died; the overall death rate was 105.7 per 1,000 person-years. Males with an AL score of 0-1 (low), 2-4 (medium), and 5-9 (high) had a death rate of 66.1, 110.4.6, and 201.3 per 1,000 person-years, respectively (Table 3).

In the unadjusted Cox model, per unit higher AL score was significantly associated with a 75% higher hazard of death among males (95% CI: 44%, 112%; Table 3). The association slightly attenuated but persisted in the full adjusted model (hazard ratio [HR] = 1.51, 95% CI: 1.23, 1.84). When modelled in categories, in the unadjusted model, the hazard of death of male with the medium AL burden (score: 2-4) was 1.68 times than hazard of death of those with a lower AL burden (score: 0-1); Male with a high AL burden (Score: 5-9) had a more than three-fold hazard of death than those with a lower AL burden (score: 0-1). These associations persisted after adjustment of socio-demographics and lifestyles. In the fully adjusted model, males with a medium AL burden (score: 2-4) had a 33% higher hazard of death than those with a low AL burden (score: 0-1). Males with a high AL burden (score: 5-9) had a more than two-fold hazard of death than those with a low AL burden (score: 0-1). Results did not change substantially in the sensitivity analyses (Tables S3).

Association between Allostatic Load and Mortality among Females

Over an average follow-up period of 3.9 years, 787 deaths (51.8%) occurred. A total of 477 females died (58.9%); the overall death rate was 161.3 per 1,000 person-years. The death rates for females with an AL score of 0-1 (low burden), 2-4 (medium burden), and 5-9 (high burden) were 121.1, 174.8, and 252.6 per 1,000 person-years, respectively (Table 3).

In the unadjusted Cox model, per unit higher AL (modelled continuously) was significantly associated with a 45% higher hazard of death among females (95% CI: 23%, 72%; Table 3). However, the association attenuated and became insignificant after adjusting for socio-demographics (age, sex, ethnicity, education, and marital status); similar results were observed when smoking, exercise, and chronic conditions including pulmonary disease and arthritis were additionally adjusted (HR = 1.16, 95% CI: 0.97, 1.38). When modelled in categories, the HR was 1.44 (95% CI: 1.17, 1.79) and 2.11 (95% CI: 1.44, 3.10) for females with an AL score of 2-4 and 5-9, respectively, compared with those with a score of 0-1 in the unadjusted model. After multivariable adjustment, females with an AL score of 2-4 and 5-9 had a 11% and 34% higher hazard of death, although the associations were not significant. Results did not change substantially in the sensitivity analyses (Tables S3).

DISCUSSION

The present study aimed to explore the association between AL burden and all-cause mortality among men and women aged at least 60 years in China. We found that older men with high AL burden had a more than two-fold hazard of death than those with a low AL burden. However, the association was less clear among women. These findings were in line with previous studies showing men tend to have higher AL with higher risk of death than women, and gender difference among AL score and cause-specific mortality risk including infectious diseases, cardiometabolic disease, and malignant neoplasm [14,26,27]. One possible

explanation for the sex difference in the association between AL and mortality among older adults is that older women might be less vulnerable to stress men due to sex difference in hippocampal formation in humans [10,16,21]. In addition, it has been shown that estrogen plays an important role in brain with the development of aging, aiming to maintain allostasis when facing physiological stress, which may be possible to protect women against age-related diseases [3,22,28]. Moreover, Gruenewald et al. (2006) stated that gender difference in forecasting mortality risk among older people by biomarkers; compared with female, neuroendocrine and immune related biomarkers were more predictive in male [12]. In addition, the sex difference in the association between AL and mortality may be explained by behavioral factors. Social support is an effective in relieving stress [40]. Women are more socially active to seek emotional support when facing stress than men [40]. Furthermore, in our study, women had high mean age, less education, lower prevalence of smoking, and less exercise. Therefore, adjustment of these covariates may influence the significant level of association in final model.

Previous study has identified increased risk of all-cause mortality associated with increasing AL score in men [14]. We found that the strength of the association differed between the present study and Hwang et al.'s work. There are several plausible explanations for this discrepancy. First, the population in Hwang et al.'s study (≥54 years) was younger than ours (≥60 years). Second, we did not include cortisol, which is a commonly used indicator of the primary mediator stress, due to data unavailability. Surrogate measures were used in the present study, which may lead to weaker associations. Third, two studies used different cut-points for constructing the AL score. We used sex-specific cut-off points for each biomarker; while general cut-points were used in Hwang et al.'s work. Fourth, follow-up length was different between the two studies.

For the sensitivity analysis of association between AL and mortality, the overall magnitude of the HR was not largely altered, and the association were still statically significant. This suggest that the results of association between AL category and mortality may not influenced by the participants who were self-reported disease status, which may support that our results are robust (Table S3).

The AL was initially constructed using primary mediator stress including cortisol, epinephrine, and norepinephrine. These biomarkers are not widely available and secondary responses of cardiovascular, inflammatory and metabolic biomarkers such as CRP, BP and heart rate were used as surrogate measures. Although multiple CVD risk factors were included, AL, in theory, represents multisystem physiological dysregulation instead of functional decline in one system. Previous studies showed that AL was able to stratify the risk of a wider range health outcomes than traditional CVD risk factors [24], it appears that AL could predict more health information including CVD incident, decline of cognition function, decline of physical function, and mortality than traditional CVD risk factors. The current study did not have data on primary mediator of AL, so we did not conduct separate analysis to investigate the association between primary vs. secondary mediators of AL and mortality.

This study has some strengths. This is among the first to investigate the association between AL and mortality using a Chinese population. Moreover, our study used of CLHLS dataset that is a large nationally representative old population survey in China. Furthermore, we updated quartile risk method for biomarkers BMI, total cholesterol, and triglyceride due to inversely association with mortality among older adults, which may more truly reflect AL score in old people. Additionally, our study added evidence to support sex difference in the association between AL and the increased risk of mortality. Finally, sex-specific cut-off points were used to construct the AL score may more truly reflect association between AL and mortality in

different gender compared previous study. Additionally, we collected detailed covariates information including age, sex, marital status, ethnicity, education, chronic diseases, which enable us to adjust for a range of potential confounders in the final cox model.

Despite these strengths, we acknowledge some limitations. First, we did not include any primary neuroendocrine biomarkers such as cortisol in constructing the AL score due to data limitation. The cortisol biomarker plays an important role in responding stress, which needs repeated measurements within 1-2 days which causes difficulties to measure in large national survey [7]. Inclusion of cortisol biomarker maybe improve our power of AL score predictions for mortality. Additionally, we classified AL biomarkers based on sex-specific quartiles; however, these measures may vary over time, leading to misclassification. Furthermore, there is huge loss to follow up (>20%; 552 of 2439), although only 3 to 7-year range of follow up (2011 to 2014-18), which may underestimate the association. Lastly, participants who provided blood sample in this study were residents in eight longevity areas including Laizhou of Shandong Province, Xiayi of Henan Province, Zhongxiang of Hubei Province, Mayang of Hunan Province, Sanshui of Guangdong Province, Yongfu of Guangxi Autonomous Region, Chengmai of Hainan Province, Rudong of Jiangsu Province, which were from 8 of 23 provinces, five autonomous regions, four municipalities, and two special administrative regions. Therefore, our results may not be greatly generalizable to older adults living in other regions of China.

Therefore, even though we found that higher AL burden was associated with increased all-cause mortality among Chinese men aged at least 60 years, but not women, it is really recommended that these results need to be replicated in large longitudinal studies with more longer follow-up time, with more AL biomarkers such as cortisol, or with containing Chinese

from more regions apart from eight longevity areas. This would helpful for comparing with our results and validating them in different population.

CONCLUSION

In conclusion, our study showed that higher AL burden was associated with increased all-cause mortality among Chinese men aged at least 60 years. We did not find strong evidence among women. Intervention programs targeting modifiable components of the AL burden may help prolong lifespan for older adults, especially men, in China.

Abbreviation:

AL: allostatic load; DHEA-S: dehydroepiandrosterone sulphate; BP: blood pressure; CLHLS: Chinese Longitudinal Healthy Longevity Survey; SBP: systolic BP; DBP: diastolic BP; BMI: body mass index; HDL: total cholesterol, high density lipoprotein; CRP: C-reactive protein.

DECLARATIONS:

Ethics approval and consent to participate:

The original CLHLS protocol was approved by the Institutional Review Board, Duke University (Pro00062871), and the Biomedical Ethics Committee, Peking University (IRB00001052-13074), and all participants have signed informed consent.

Consent to publish:

Not required

Availability of data and materials:

The raw data are available on website:

https://opendata.pku.edu.cn/dataset.xhtml?persistentId=doi:10.18170/DVN/WBO7LK

Competing interests:

Dr. Chenkai Wu provides paid consultant services to Health Keepers, a start-up health data analytics company in China. Other authors have no conflict of interests to disclose.

Funding:

The data analyzed in this paper were provided by the Chinese Longitudinal Healthy Longevity Study (CLHLS) which has been jointly supported by National Natural Sciences Foundation of

China (71490732) and the National Key R&D Program of China (2018YFC2000400); Ministry of Science and Technology in China (2020YFC2005600); Suzhou Municipal Science and Technology Bureau (SS2019069). The research results of this article are sponsored partly by the Kunshan Municipal Government research funding.

The funder played no role in study design, data collection, and analysis, the decision to publish, or preparation of the manuscript.

Author Contributions:

THZ conceived of and conducted the data analyses, interpreted the findings, and wrote the manuscript. LJY, HSC and HYJ contributed to manuscript revision. CKW contributed to analysis and interpretation of data and drafting and revision of the manuscript.

Acknowledgements:

Not Applicable

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Table 1.

Cut-point for each of nine biomarkers used to construct allostatic load.

	Cut-points	
Biomarkers	**Male**	**Female**
Body mass index, kg/m^2	≤19.33	≤17.78
Glucose, mmol/L	≥5.13	≥5.15
Total cholesterol, mmol/L	≤3.51	≤3.71
High-density lipoprotein cholesterol, mmol/L	≤1.04	≤1.06
Triglyceride, mmol/L	≤0.56	≤0.63
High-sensitive C reactive protein, mg/L	≥2.44	≥2.33
Heart rate, beats/min	≥80	≥83
Systolic blood pressure, mmHg	≥150	≥160
Diastolic blood pressure, mmHg	≥90	≥90

High-risk group was defined as below the sex-specific 25th percentile for body mass index, total cholesterol, high-density lipoprotein cholesterol and triglyceride. High-risk group was defined as above the sex-specific 75th percentile for glucose, high-sensitive C reactive protein, heart rate, systolic blood pressure, and diastolic blood pressure.

Table 2.

Baseline characteristics by allostatic load burden (low, medium, and high) among males and females.

	Male (N=709)					Female (N=810)				
	Total	Low (n=181)	Medium (n=455)	High (n=73)	P-value	Total	Low (n=231)	Medium (n=534)	High (n=45)	P-value
Age, years, mean±SD	80.5±11.3	77.6±10.7	81.0±11.4	84.1±11.0	0.042	90.2±11.8	87.0±13.0	91.3±11.1	93.6±11.0	0.029
Married, No. (%)	446 (62.9%)	125 (69.1%)	276 (60.7%)	45 (61.6%)	0.137	173 (21.4%)	65 (28.1%)	101 (18.9%)	7 (15.6%)	0.010
Han ethnicity, No. (%)	638 (89.9%)	168 (92.8%)	402 (88.4%)	68 (93.2%)	0.152	719 (88.8%)	210 (90.9%)	471 (88.2%)	38 (84.4%)	0.354
Any education, No. (%)	457 (64.5%)	119 (65.8%)	294 (64.6%)	44 (60.3%)	0.707	108 (13.3%)	50 (21.7%)	55 (10.3%)	3 (6.7%)	0.000
Smoking, No. (%)					0.418					0.800
Current Smoker	265 (37.3%)	71 (39.2%)	170 (37.4%)	24 (32.9%)		30 (3.7%)	6 (2.6%)	23 (4.3%)	1 (2.2%)	
Past Smoker	109 (15.4%)	22 (12.2%)	71 (15.6%)	16 (21.9%)		16 (2.0%)	5 (2.2%)	11 (2.1%)	0	
Non-smoker	335 (47.3%)	88 (48.6%)	214 (47.0%)	33 (45.2%)		761 (94.3%)	219 (95.2%)	498 (93.6%)	44 (97.8%)	
Exercise, No. (%)	183 (26.2%)	55 (30.6%)	110 (24.6%)	18 (25.0%)	0.300	154 (19.3%)	41 (18.1%)	103 (19.5%)	10 (22.2%)	0.782
Hypertension, No. (%)	125 (17.8%)	0	97 (21.6%)	28 (38.9%)	0.000	179 (22.6%)	0	153 (29.3%)	26 (57.8%)	0.000
Diabetes, No. (%)	13 (1.8%)	3 (1.6%)	4 (0.9%)	6 (8.3%)	0.001	12 (1.5%)	0	10 (1.9%)	2 (4.4%)	0.013
Heart disease, No. (%)	42 (5.9%)	10 (5.5%)	27 (6.0%)	5 (6.9%)	0.921	62 (7.8%)	10 (4.5%)	40 (7.6%)	12 (26.7%)	0.000
Stroke, No. (%)	33 (4.7%)	8 (4.4%)	18 (4.0%)	7 (10.0%)	0.105	45 (5.7%)	14 (6.2%)	29 (5.5%)	2 (4.4%)	0.878
Pulmonary, No. (%)	74 (10.5%)	18 (10.0%)	45 (9.9%)	11 (15.3%)	0.374	49 (6.2%)	10 (4.4%)	38 (7.2%)	1 (2.2%)	0.181
Arthritis, No. (%)	96 (13.6%)	18 (10.0%)	64 (14.2%)	14 (19.2%)	0.132	128 (16.0%)	44 (19.2%)	79 (15.0%)	5 (11.1%)	0.224
Cancer, No. (%)	2 (0.3%)	1 (0.6%)	1 (0.2%)	0	0.591	6 (0.8%)	0	5 (1.0%)	1 (2.2%)	0.133

[a]Married vs. widowed, never married, and divorce.

Table 3.
Association between allostatic load and mortality among males and females.

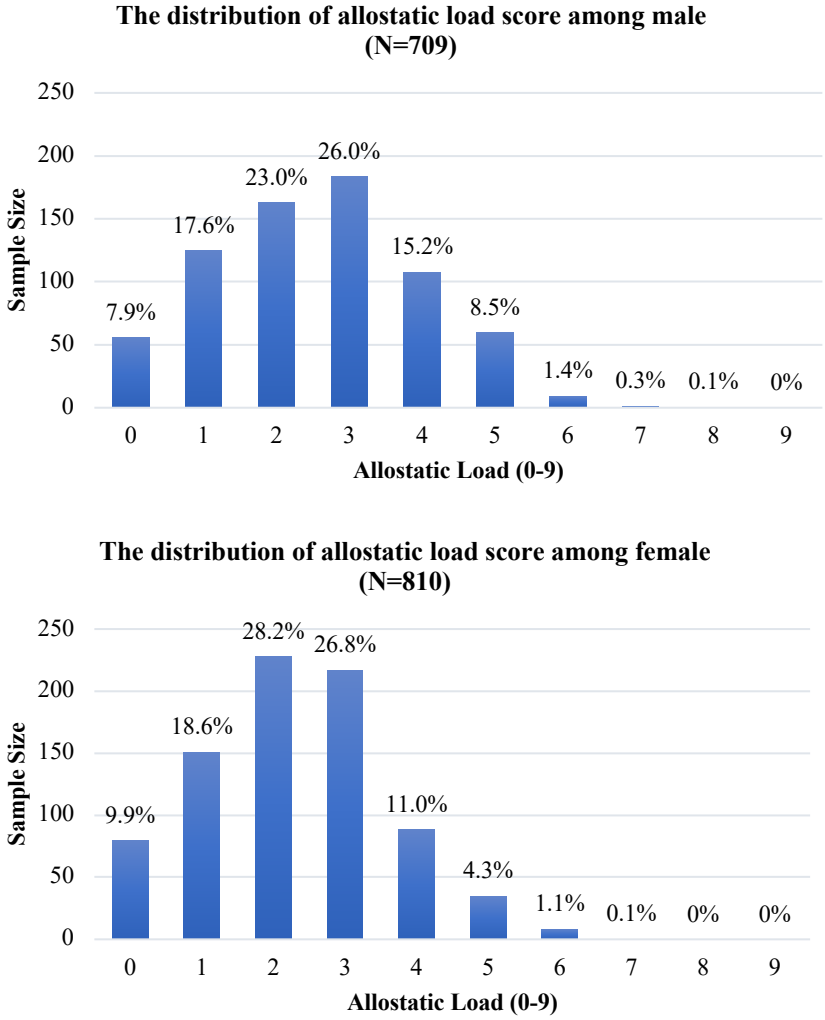
		Males (N = 709)		
	Events per 1,000 PYs (95% CI)	Unadjusted	Demographically adjusted [a]	Fully adjusted [b]
		Hazard ratio (95% CI)		
AL (continuously)		1.75 (1.44, 2.12)	1.51 (1.24, 1.84)	1.51 (1.23, 1.84)
AL category				
Low (0-1)	66.1 (50.7, 86.1)	Ref.	Ref.	Ref.
Medium (2-4)	110.4 (96.3, 126.7)	1.68 (1.24, 2.26)	1.36 (1.00, 1.83)	1.33 (0.98, 1.80)
High (5-9)	201.3 (153.0, 265.0)	3.06 (2.09, 4.48)	2.29 (1.56, 3.37)	2.18 (1.48, 3.22)
		Females (N = 810)		
	Events per 1,000 PYs (95% CI)	Unadjusted	Demographically adjusted [a]	Fully adjusted [b]
		Hazard ratio (95% CI)		
AL (continuously)		1.45 (1.23, 1.72)	1.16 (0.97, 1.37)	1.16 (0.97, 1.38)
AL category				
Low (0-1)	121.1 (100.7, 145.6)	Ref.	Ref.	Ref.
Medium (2-4)	174.8 (156.9, 194.7)	1.44 (1.17, 1.79)	1.14 (0.92, 1.42)	1.11 (0.89, 1.38)
High (5-9)	252.6 (180.5, 353.5)	2.11 (1.44, 3.10)	1.36 (0.93, 2.01)	1.34 (0.91, 1.97)

Abbreviations: PY, person-year; CI, confidence interval; AL, allostatic load.

[a]Demographically adjusted model included age, ethnicity (Han vs. minority), education (any vs. none), and marital status (married vs. others).

[b]Fully adjusted model included age, ethnicity (Han vs. minority), education (any vs. none), and marital status (married vs. others), smoking (current vs. previous or non-smoke), exercise (yes vs. no), and chronic diseases such as pulmonary disease and arthritis.

Figure 1.
Distribution of sample size by allostatic load score among males and females.



The above figure is the distribution of allostatic load score among male. The below figure is the distribution of allostatic load score among female. The distribution of the AL score (range: 0-9) is right skewed for both males and females; only 13 (1.8%) males and 10 (1.2%) females had a score of 6-9, respectively.

Table S1.
Baseline characteristics between included and excluded participants.

Characteristics	Included (N=1,519)	Excluded [b] (N=920)	P-value
Age, years, mean±SD	85.6±12.6	85.6± 13.5	0.523
Married [a], No. (%)	619 (40.8%)	361 (39.2%)	0.461
Han ethnicity, No. (%)	1,357 (89.3%)	836 (90.9%)	0.223
Any education, No. (%)	565 (37.2%)	384 (41.7%)	0.026
Smoking, No. (%)			0.460
Current Smoker	295 (19.5%)	170 (18.7%)	
Past Smoker	125 (8.3%)	88 (9.7%)	
Non-smoker	1,096 (72.3%)	650 (71.6%)	
Exercise, No. (%)	337 (22.5%)	271 (30.2%)	0.000
Hypertension, No. (%)	304 (20.3%)	201 (22.8%)	0.153
Diabetes, No. (%)	25 (1.7%)	19 (2.1%)	0.405
Heart disease, No. (%)	104 (6.9%)	48 (5.4%)	0.138
Stroke, No. (%)	78 (5.2%)	62 (6.9%)	0.077
Pulmonary, No. (%)	123 (8.2%)	73 (8.2%)	0.994
Arthritis, No. (%)	224 (14.9%)	152 (17.1%)	0.145
Cancer, No. (%)	8 (0.5%)	3 (0.3%)	0.341

[a]Married vs. widowed, never married, and divorced.

[b]Excluded criteria includes incomplete data on AL biomarkers, no follow-up data, missing data or extreme values of the biomarkers, and aged less than 60 years.

Table S2.

The cut-points for excluding SBP, DBP, BMI, and heart rate.

Biomarkers	Cut-points
Body mass index, kg/m^2	>40
Heart rate, beats/min	>220
Systolic blood pressure, mmHg	>200 or <90
Diastolic blood pressure, mmHg	>110 or <60

Table S3.
Sensitivity analysis.

		Males (N = 709)		
	Events per 1,000 PYs (95% CI)	Unadjusted	Demographically adjusted [a]	Fully adjusted [b]
		Hazard ratio (95% CI)		
AL (continuously)		1.28 (1.18, 1.38)	1.18 (1.09, 1.28)	1.18 (1.08, 1.27)
AL category				
Low (0-1)	68.1 (53.4, 86.9)	Ref.	Ref.	Ref.
Medium (2-4)	115.4 (100.7, 132.1)	1.70 (1.29, 2.25)	1.45 (1.10, 1.93)	1.41 (1.06, 1.88)
High (5-9)	214.7 (154.8, 297.6)	3.18 (2.11, 4.78)	2.33 (1.54, 3.53)	2.35 (1.55, 3.56)
		Females (N = 810)		
	Events per 1,000 PYs (95% CI)	Unadjusted	Demographically adjusted [a]	Fully adjusted [b]
		Hazard ratio (95% CI)		
AL (continuously)		1.22 (1.14, 1.31)	1.10 (1.02, 1.18)	1.09 (1.01, 1.17)
AL category				
Low (0-1)	113.7 (96.2 134.4)	Ref.	Ref.	Ref.
Medium (2-4)	191.7 (171.8, 213.9)	1.70 (1.39, 2.08)	1.29 (1.05, 1.57)	1.27 (1.03, 1.56)
High (5-9)	267.4 (168.5, 424.5)	2.40 (1.47, 3.93)	1.37 (0.83, 2.25)	1.39 (0.85, 2.29)

Abbreviations: PY, person-year; CI, confidence interval; AL, allostatic load.

[a]Demographically adjusted model included age, ethnicity (Han vs. minority), education (any vs. none), and marital status (married vs. others).

[b]Fully adjusted model included age, ethnicity (Han vs. minority), education (any vs. none), and marital status (married vs. others), smoking (current vs. previous or non-smoke), exercise (yes vs. no), and chronic diseases such as pulmonary disease and arthritis.

Table S4.
Compare participant who lost follow up data and who included in study.

Characteristics	Included (N=1,519)	Loss follow up (N=522)	P-value
Age, years, mean±SD	85.6±12.6	87.3± 13.3	0.98
Married [a], No. (%)	619 (40.8%)	180 (34.8%)	0.02
Han ethnicity, No. (%)	1,357 (89.3%)	467 (90.2%)	0.60
Any education, No. (%)	565 (37.2%)	202 (39.0%)	0.47
Smoking, No. (%)			
Current Smoker	295 (19.5%)	88 (17.3%)	0.54
Past Smoker	125 (8.3%)	45 (8.8%)	
Non-smoker	1,096 (72.3%)	376 (73.9%)	
Exercise, No. (%)	337 (22.5%)	129 (25.6%)	0.15
Hypertension, No. (%)	304 (20.3%)	109 (21.9%)	0.45
Diabetes, No. (%)	25 (1.7%)	7 (1.4%)	0.68
Heart disease, No. (%)	104 (6.9%)	25 (5.0%)	0.13
Stroke, No. (%)	78 (5.2%)	44 (8.8%)	0.004
Pulmonary, No. (%)	123 (8.2%)	39 (7.7%)	0.77
Arthritis, No. (%)	224 (14.9%)	79 (15.8%)	0.62
Cancer, No. (%)	8 (0.5%)	2 (0.4%)	0.72

[a]Married vs. widowed, never married, and divorced.

zSTROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	1-2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	5-6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up (*b*) For matched studies, give matching criteria and number of exposed and unexposed	6-7
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7-9
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7-9
Bias	9	Describe any efforts to address potential sources of bias	7
Study size	10	Explain how the study size was arrived at	6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7-9
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding (*b*) Describe any methods used to examine subgroups and interactions (*c*) Explain how missing data were addressed (*d*) If applicable, explain how loss to follow-up was addressed (*e*) Describe any sensitivity analyses	9
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (b) Give reasons for non-participation at each stage (c) Consider use of a flow diagram	10
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (b) Indicate number of participants with missing data for each variable of interest (c) Summarise follow-up time (eg, average and total amount)	10
Outcome data	15*	Report numbers of outcome events or summary measures over time	11-12

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (*b*) Report category boundaries when continuous variables were categorized (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	11-12
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	11-12
Discussion			
Key results	18	Summarise key results with reference to study objectives	12
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	15
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12-14
Generalisability	21	Discuss the generalisability (external validity) of the study results	15
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	17-18

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

BMJ Open

The Association between Allostatic Load and Mortality among Chinese Older Adults: The Chinese Longitudinal Health and Longevity Study

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2020-045369.R1
Article Type:	Original research
Date Submitted by the Author:	27-Mar-2021
Complete List of Authors:	Zhang, Tianhang; Duke Kunshan University, Global Health Research Center; Imperial College London Yan, Lijing L; Duke Kunshan University, Global Health Research Center; Duke Global Health Institute Chen, Hua-Shuai; Duke University, Jin, Hai-Yu; Duke Kunshan University, Global Health Research Center wu, chenkai; Duke Kunshan University, Global Health Research Center
Primary Subject Heading:	Epidemiology
Secondary Subject Heading:	Geriatric medicine, Epidemiology
Keywords:	MENTAL HEALTH, PUBLIC HEALTH, EPIDEMIOLOGY

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The Association between Allostatic Load and Mortality among Chinese Older Adults: The Chinese Longitudinal Health and Longevity Study

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Word count: 3736

The Association between Allostatic Load and Mortality among Chinese Older Adults: The Chinese Longitudinal Health and Longevity Study

Abstract

Background: Allostatic load has shown that high burden of AL is associated with increased risk of adverse outcomes, but little attention has been paid to China with largest aging population in the world.

Objective: This study is to examine the association between allostatic load (AL) and all-cause mortality among Chinese adults aged at least 60 years.

Design: Population-based prospective cohort study.

Setting: In 2011-2012, an ancillary study, in which a blood test was added, including a total of 2,439 participants, was conducted in eight longevity areas in the Chinese Longitudinal Healthy Longevity Survey.

Participants: The final analytic sample consisted of 1,519 participants (mean ± SD age: men 80.5±11.3 years; women 90.2±11.8 years; and 53% women).

Primary outcome measure: Cox models were used to examine the association between AL and mortality among men and women, separately. Analysis were also adjusted for potential confounders including age, ethnicity, education, and marital status, smoking and exercise.

Results: Male with a medium AL burden (score: 2-4) and high AL burden (score: 5-9) had a 33% and 118% higher hazard of death, respectively, than those with a low AL burden (score: 0-1). We did not find significant difference between females with different levels of AL burden.

Conclusion: Higher AL burden was associated with increased all-cause mortality among Chinese men aged at least 60 years. However, we did not find strong association among women. In conclusion, Intervention programs targeting modifiable components of the AL burden may help prolong lifespan for older adults, especially men, in China.

Keywords: Allostatic load; Mortality; Chinese; Elderly; Cohort study; disease burden.

ARTICLE SUMMARY

Strengths and limitations of this study

- This is the first study to investigate the association between AL and mortality using a Chinese population.
- The CLHLS dataset that is a large nationally representative old population survey in China.
- The updated quartile risk method for biomarkers BMI, total cholesterol, and triglyceride among older adults.
- Lack of primary neuroendocrine biomarkers such as cortisol in constructing the AL score, which may influence the finding presented.
- There is huge loss to follow up (>20%; 552 of 2439), although only 3 to 7-year range of follow up (2011 to 2014-18), which may underestimate the association.

The Association between Allostatic Load and Mortality among Chinese Older Adults: The Chinese Longitudinal Health and Longevity Study

BACKGROUND

Allostatic load (AL) is conceptualized as the cumulative wear and tear on multiple physiological systems resulting from repeated adaptation to stressors [1,2,3,4]. In the absence of a gold standard, many operational definitions of AL have been proposed. The most commonly used construct of AL was developed by Seeman and colleagues who have used two categories of biomarkers for quantifying AL [4,5]. The first category (called primary mediators) includes biomarkers the body releases in response to stress, such as cortisol and dehydroepiandrosterone sulphate (DHEA-S); the second category comprises secondary outcomes that result from the effects of primary mediators. Examples of biomarkers are blood pressure (BP), cholesterol, and the waist-hip ratio [4].

A number of studies identified that a high burden of AL is associated with increased risk of adverse outcomes including cardiovascular disease, functional decline, and mortality among the older adults [1,2,6,7,8,9,10]. For example, in a 7-year longitudinal study conducted in 2006, increased AL score was associated with higher mortality among older population [8]. In a cohort study of 1,023 community-dwelling older adults in Taiwan, researchers found that higher AL score was related with higher death rate [7]. Additionally, some studies found that women and men experienced chronic stress in different ways. For example, Yang et al in 2011 revealed gender differences in the AL biomarkers and the age trajectories of physiological dysregulation [11]. Women had a higher level of inflammation biomarkers but lower risk of cardiovascular disease (CVD) than men. Another study from Tampubolon and Maharani in 2018 among the older population found that AL score increased in sex difference [12].

Compared with men, women showed an advantage in life expectancy [12]. Taken together, these results suggest the use of sex-specific cut-off points for AL biomarkers in future research.

A number of studies have examined the association between AL and mortality among older adults. However, little attention has been paid to less developed regions, including China – the most populous country with the largest aging population in the world. In 2019, there were 249 million adults aged 60 years or above in China, accounting for 17.3% of its total population, and this number is projected to almost double in 2050, reaching 487 million [13,14]. Understanding the relationship between AL and mortality in less developed countries is beneficial for leading to interventions, which could be helpful to change unhealthy lifestyles, decrease morbidity and mortality among the older population. In addition, less studies focus on sex-specific cut off points for calculating AL index, this study will conduct sex-specific studies, which may closely reflect AL score among the older population.

In this study, we used a large cohort study to examine the association between AL and all-causes mortality among Chinese men and women aged at least 60 years. We hypothesized that a higher burden of AL would be associated with increased risk of all-cause mortality among both older men and women in China.

METHODS

Data and Study Participants

We used data from the Chinese Longitudinal Healthy Longevity Survey (CLHLS), an ongoing prospective, longitudinal study with the largest sample of the oldest old in China. Half of the counties and cities in 22 of the 31 provinces in China (covering 85% of the population) were randomly selected through a multistage cluster sampling approach. A wide range of socio-

demographic, lifestyle, and health measures were collected in the CLHLS. The baseline survey was conducted in 1998 and participants who were alive were re-interviewed in each follow-up survey (2000, 2002, 2005, 2008-2009, 2011-2012, 2014, and 2017-2018). In 2011-2012, an ancillary study, in which a blood test was added, was conducted in eight longevity areas: Laizhou City in Shandong Province, Xiayi County in Henan Province, Zhongxiang City in Hubei Province, Mayang County in Hunan Province, Yongfu County in Guangxi Autonomous Area, Sanshui District in Guangdong Province, Chengmai County in Hainan Province, and Rudong County in Jiangsu Province. The Research Ethics Committees of Peking University and Duke University granted approval for the Protection of Human Subjects for the CLHLS. All study participants gave informed consent. A more detailed description of the recruitment strategy and study design of the CLHLS has been published elsewhere [15,16,17]. A total of 8,959 individuals were included at baseline (1998). 1998 baseline survey, which was extended to 11,162 in 2000, it was found that almost 30 percent died before 2002 interview and approximately 14 percent were lost that was higher than the attrition rate between 1998 and 2000 wave (9.6 percent); the number of participants were extend to 16,064 in 2002, and about 13.8 percent were lost between 2002 and 2005; the number of interviewed were 15,638 in 2005, and about 13.2 percent were lost between 2005 and 2008-2009; the number of participants were extended to 16.540, and approximately 17.7 percent were lost between 2008-2009 and 2011-2012; the total number of interviewed participants were 9765 in 2011-2012 [18].

A total of 2,439 persons contributed blood samples in the ancillary study (2011-2012). Participants were excluded from the analytic sample if they had (i) incomplete data on any biomarkers for constructing AL (n = 251), (ii) no follow-up data (time to death or censorship was undetermined; n = 552), (iii) had extreme values on the biomarkers (n = 109; Table S1), or (iv) were less than 60 years old (n=16). The final analytic sample consisted of 1,519

participants. We did not observe appreciable differences in age, ethnicity, marital status, smoking, or chronic conditions between the analytic sample and those excluded (n = 920; Table S2). Compared to the analytic sample, excluded persons had a higher education level and higher prevalence of exercise. In addition, compared to people who were lost follow up, the included people had higher prevalence of married, and higher prevalence of stroke; we did not observe appreciable differences in age, ethnicity, smoking, exercise, hypertension, diabetes, heart disease, pulmonary, arthritis, and cancer between both (Table S3).

Calculation of AL Score

Based on previous research [3,7,19,20] and availability of data in the CLHLS, we selected nine biomarkers to construct AL: heart rate, systolic BP (SBP), and diastolic BP (DBP), body mass index (BMI), total cholesterol, high-density lipoprotein (HDL) cholesterol, glucose, triglyceride, and C-reactive protein (CRP). BMI, heart rate, SBP, and DBP were collected from physical examinations. BMI was calculated as body weight (kilograms) divided by height (meters) squared. SBP and DBP were measured by a mercury sphygmomanometer with an appropriately sized cuff, taken in the seated position after 5 minutes of quiet rest under the supervision of trained research assistants. We used the average of two measurements for further analyses. Blood samples were used for assays of the level of the total cholesterol, HDL cholesterol, glucose, triglyceride, and CRP.

To be in line with previous studies [4,10,21,22], we used the highest quartile for heart rate, SBP, DBP, glucose, and CRP and the lowest quartile for HDL cholesterol to define the high-risk group (coded 1). Because BMI, total cholesterol, and triglyceride were inversely associated with mortality among older adults, especially the oldest old [23, 24, 25], we used the lowest quartile to define the high-risk group for these three biomarkers. For participants who self-

reported having been diagnosed with hypertension and heart disease, we classified their SBP, DBP, and glucose into the high-risk category. Similarly, we classified participants' glucose into the high-risk group if they self-reported having been diagnosed with diabetes. The identification of risk quartiles of biomarkers is commonly used to construct AL index [5, 26]. The cut-points of all nine AL components by men and women were presented in Table 1. We constructed the AL score based on the count of biomarkers falling in the high-risk group, ranging from 0 (lowest) to 9 (highest). To be in line with previous studies [27], we then considered using similar cut-off points, classifying the AL score into three categories based on sample distribution: 0-1 (low burden), 2-4 (medium burden), and 5-9 (high burden).

Mortality

The outcome was all-cause mortality. Vital status and date of death (for persons who died by the end of the study) was ascertained by the close family member or village doctor of the deceased participant during the follow-up survey in 2014 and 2017-2018. We calculated the survival time from the date of the baseline interview to the date of last interview (censored) or the death date.

Covariates

Demographic and lifestyle characteristics were collected by interview, including age, sex, ethnicity, education, and marital status, smoking status, and physical exercise. We divided ethnicity into Han and others (minority groups). Years of education were dichotomized as any (one year or more) and no education, which is commonly way used in CLHLS study [28, 29]. Marital status was dichotomized as married and others (widowed, not married, and divorced). Cigarette smoking was categorized as current, past, and non-smoker. Information of exercise was collected using the question "Do you do exercise at present?" and dichotomized into yes

or no. Chronic conditions were measured based on self-reported physician's diagnosis, including hypertension, diabetes, heart disease, stroke, pulmonary disease (including bronchitis, emphysema, pneumonia and asthma), arthritis, and cancer.

Statistical Analyses

All analyses were conducted separately for males and females. We first presented the relative frequency of the AL score using histograms and calculated the mean AL score. Then, we described the baseline characteristics of the study sample by AL burden (low, medium, and high) using means and SDs for continuous variables and counts and percentages for categorical variables. Characteristics were compared across the three AL categories using analyses of variance for continuous variables and chi-square tests for categorical variables.

We calculated the death rates across three AL categories (low, medium, and high burden). We used the Cox proportional hazards model to determine the unadjusted and adjusted associations between the AL and all-cause mortality. Age, sex, education, and marital status were included in the demographically adjusted models; smoking status, physical exercise, and chronic status including pulmonary disease and arthritis were added in the fully adjusted models. We modeled AL both continuously and in categories.

Furthermore, one sensitivity analysis was undertaken, which aimed to exam if the model results were influenced when we did not use self-reported hypertension or diabetes to classify participants' risk category for BP or glucose.

All tests were two-sided with a significance level of P-value less than 0.05. We conducted all analyses using STATA version 16.0 (Stata Corp, College Station, TX).

Study participants and public involvement

This research was done without study participant involvement. Study participants were not invited to comment on the study design and were not consulted to develop participant-relevant outcomes or interpret the results. Participants were not invited to contribute to the writing or editing of this document for readability or accuracy.

RESULTS

Distribution of Allostatic Load Categories

The distribution of the AL score (range: 0-9) is right-skewed for both males and females; only 13 (1.8%) males and 10 (1.2%) females had a score of 6-9, respectively (Figure 1). The mean AL score was 2.56 (SD=1.47) for males and 2.28 (SD=1.34) for females. For males, 25.5%, 64.2%, and 10.3% had an AL score of 0-1, 2-4, and 5-9, respectively. For females, 28.5%, 65.9%, and 5.6% had an AL score of 0-1 (low burden), 2-4 (medium burden), and 5-9 (high burden), respectively.

Demographic Characteristics

A total of 709 (46.7%) males were included. The average age for males with an AL score of 0-1 (low burden), 2-4 (medium burden), 5-9 (high burden) was 77.6, 81.0, and 84.1 years, respectively (P = 0.042). In addition, we observed significant differences in the prevalence hypertension and diabetes by different AL burden among males.

The study sample included 810 (53.3%) females. The average age for females with an AL score of 0-1 (low burden), 2-4 (medium burden), 5-9 (high burden) was 87.0, 91.3, and 93.6 years, respectively (P < 0.05; Table 2). Compared with men, women were older, less educated, had a

lower prevalence of smoking, and were less physically active. Females with a lower AL were more likely to be married and have any education than woman with higher AL score; they also had a lower prevalence of hypertension, diabetes, and heart disease. We did not observe significant difference in ethnicity, smoking, physical exercise, stroke, pulmonary disease, arthritis, and cancer across AL burden (low, medium, and high) among females.

Association between Allostatic Load and Mortality among Males

A total of 310 males died; the overall death rate was 105.7 per 1,000 person-years. Males with an AL score of 0-1 (low), 2-4 (medium), and 5-9 (high) had a death rate of 66.1, 110.4.6, and 201.3 per 1,000 person-years, respectively (Table 3).

In the unadjusted Cox model, per unit higher AL score was significantly associated with a 75% higher hazard of death among males (95% CI: 44%, 112%; Table 3). The association slightly attenuated but persisted in the full adjusted model (hazard ratio [HR] = 1.51, 95% CI: 1.23, 1.84). When modelled in categories, in the unadjusted model, the hazard of death of male with the medium AL burden (score: 2-4) was 1.68 times than hazard of death of those with a lower AL burden (score: 0-1); Male with a high AL burden (Score: 5-9) had a more than three-fold higher hazard of death than those with a lower AL burden (score: 0-1). These associations persisted after adjustment of socio-demographics and lifestyles. In the fully adjusted model, males with a medium AL burden (score: 2-4) had a 33% higher hazard of death than those with a low AL burden (score: 0-1). Males with a high AL burden (score: 5-9) had a more than two-fold higher hazard of death than those with a low AL burden (score: 0-1). Results did not change substantially in the sensitivity analyses (Tables S4).

Association between Allostatic Load and Mortality among Females

Over an average follow-up period of 3.9 years, 787 deaths (51.8%) occurred. A total of 477 females died (58.9%); the overall death rate was 161.3 per 1,000 person-years. The death rates for females with an AL score of 0-1 (low burden), 2-4 (medium burden), and 5-9 (high burden) were 121.1, 174.8, and 252.6 per 1,000 person-years, respectively (Table 3).

In the unadjusted Cox model, per unit higher AL (modelled continuously) was significantly associated with a 45% higher hazard of death among females (95% CI: 23%, 72%; Table 3). However, the association attenuated and became insignificant after adjusting for socio-demographics (age, sex, ethnicity, education, and marital status); similar results were observed when smoking, exercise, and chronic conditions including pulmonary disease and arthritis were additionally adjusted (HR = 1.16, 95% CI: 0.97, 1.38). When modelled in categories, the HR was 1.44 (95% CI: 1.17, 1.79) and 2.11 (95% CI: 1.44, 3.10) for females with an AL score of 2-4 and 5-9, respectively, compared with those with a score of 0-1 in the unadjusted model. After multivariable adjustment, females with an AL score of 2-4 and 5-9 had a 11% and 34% higher hazard of death, although the associations were not significant. Results did not change substantially in the sensitivity analyses (Tables S4).

DISCUSSION

The present study aimed to explore the association between AL burden and all-cause mortality among men and women aged at least 60 years in China. This finding is somewhat consistent with previous evidence suggesting that AL may be predictor of all-cause mortality later in life [7,19]. In addition, we found that older men with high AL burden had a more than two-fold hazard of death than those with a low AL burden. There is no significant association observing among females, but the findings are trending in the expected direction. These findings were in line with previous studies showing men tend to have higher AL with higher risk of death than

women, and gender difference among AL score and cause-specific mortality risk including infectious diseases, cardiometabolic disease, and malignant neoplasm [7, 30, 31]. One possible explanation for the sex differences in the association between AL and mortality among older adults is that older women might be less vulnerable to stress men due to sex differences in the hippocampal formation in humans [32, 33, 34]. In addition, it has been shown that estrogen plays an important role in the brain with the development of aging, aiming to maintain allostasis when facing physiological stress, which may be possible to protect women against age-related diseases [35,36,37]. Moreover, Gruenewald et al. (2006) stated that gender difference in forecasting mortality risk among older people by biomarkers; compared with female, neuroendocrine and immune related biomarkers were more predictive in males [38]. In addition, the sex difference in the association between AL and mortality may be explained by behavioral factors. Social support is effective in relieving stress [39]. Women are more socially active to seek emotional support when facing stress than men [39]. Furthermore, in our study, women had high mean age, less education, lower prevalence of smoking, and less exercise. Therefore, adjustment of these covariates may influence the significant level of association in the final model.

To our knowledge, our study was the first to reveal a significant association between AL and mortality among male participants only. However, we need to interpret these results with caution because the findings regarding the association between AL and mortality among females were trending in the expected direction. A study with more female participants is needed to provide a more definite conclusion. Previous study has identified increased risk of all-cause mortality associated with increasing AL score in men [7]. We found that the strength of the association differed between the present study and Hwang et al.'s work among men. There are several plausible explanations for this discrepancy. First, the population in Hwang et

al.'s study (≥54 years) was younger than ours (≥60 years). Second, we did not include cortisol, which is a commonly used indicator of the primary mediator stress, due to data unavailability. Surrogate measures were used in the present study, which may lead to weaker associations. Third, two studies used different cut-points for constructing the AL score. We used sex-specific cut-off points for each biomarker, while general cut-points were used in Hwang et al.'s work. Fourth, follow-up length was different between the two studies. Furthermore, different choices of model covariates may influence the strength of observed associations. Hwang et al.'s research only adjusted for age and sex.

For the sensitivity analysis of the association between AL and mortality, the overall magnitude of the HR was not largely altered, and the association was still statically significant. This suggests that the results of the association between AL category and mortality may not influenced by the participants who were self-reported disease status, which may support that our results are robust (Table S4).

The AL was initially constructed using primary mediator stress including cortisol, epinephrine, and norepinephrine. These biomarkers are not widely available and secondary responses of cardiovascular, inflammatory and metabolic biomarkers such as CRP, BP and heart rate were used as surrogate measures. Although multiple CVD risk factors were included, AL, in theory, represents multisystem physiological dysregulation instead of functional decline in one system. Previous studies showed that AL was able to stratify the risk of a wider range of health outcomes than traditional CVD risk factors [4], it appears that AL could predict more health information including CVD incident, decline of cognition function, decline of physical function, and mortality than traditional CVD risk factors. The current study did not have data

on primary mediator of AL, so we did not conduct separate analysis to investigate the association between primary vs. secondary mediators of AL and mortality.

This study has some strengths. This is among the first to investigate the association between AL and mortality using a Chinese population. Moreover, our study used of CLHLS dataset that is a large nationally representative old population survey in China. Furthermore, we updated the quartile risk method for biomarkers BMI, total cholesterol, and triglyceride due to inversely association with mortality among older adults, which may more truly reflect AL score in old people. Additionally, our study added evidence to support sex differences in the association between AL and the increased risk of mortality. Finally, sex-specific cut-off points were used to construct the AL score may more truly reflect association between AL and mortality in different gender compared to previous study. Additionally, we collected detailed covariates information including age, sex, marital status, ethnicity, education, chronic diseases, which enable us to adjust for a range of potential confounders in the final cox model.

Despite these strengths, we acknowledge some limitations. First, we did not include any primary neuroendocrine biomarkers such as cortisol in constructing the AL score due to data unavailability; this might partially explain the null finding regarding the association between AL and mortality among women. The cortisol biomarker plays an important role in responding stress, which needs repeated measurements within 1-2 days which causes difficulties to measure in large national survey [40]. Inclusion of cortisol biomarker maybe improve our power of AL score predictions for mortality. Additionally, we classified AL biomarkers based on sex-specific quartiles; however, these measures may vary over time, leading to misclassification. Furthermore, there is huge loss to follow up (>20%; 552 of 2439), although only 3 to 7-year range of follow up (2011 to 2014-18), which may underestimate the association.

Then, participants who provided blood sample in this study were residents in eight longevity areas including Laizhou of Shandong Province, Xiayi of Henan Province, Zhongxiang of Hubei Province, Mayang of Hunan Province, Sanshui of Guangdong Province, Yongfu of Guangxi Autonomous Region, Chengmai of Hainan Province, Rudong of Jiangsu Province, which were from 8 of 23 provinces, five autonomous regions, four municipalities, and two special administrative regions. Therefore, our results may not be greatly generalizable to older adults living in other regions of China. Lastly, it is important to notice that the sample was extremely old, which may influence the magnitude of the association presented.

Therefore, even though we found that higher AL burden was associated with increased all-cause mortality among Chinese men aged at least 60 years, but not women, it is really recommended that these results need to be replicated in large longitudinal studies with longer follow-up time, with more AL biomarkers such as cortisol, or with containing Chinese from more regions apart from eight longevity areas. This would helpful for comparing with our results and validating them in different populations.

CONCLUSION

In conclusion, our study showed that higher AL burden was associated with increased all-cause mortality among Chinese men aged at least 60 years. We did not find strong evidence among women. Intervention programs targeting modifiable components of the AL burden may help prolong lifespan for older adults, especially men, in China.

Abbreviation:

AL: allostatic load; DHEA-S: dehydroepiandrosterone sulphate; BP: blood pressure; CLHLS: Chinese Longitudinal Healthy Longevity Survey; SBP: systolic BP; DBP: diastolic BP; BMI: body mass index; HDL: total cholesterol, high density lipoprotein; CRP: C-reactive protein.

DECLARATIONS:

Ethics approval and consent to participate:

The original CHARLS was approved by the Ethical Review Committee of Peking University. The baseline data collection was obtained from the Biomedical Ethics Review Committee of Peking University (IRB00001052-11015), and all participants have signed informed consent.

Consent to publish:

Not required

Availability of data and materials:

The raw data are available on website:

https://opendata.pku.edu.cn/dataset.xhtml?persistentId=doi:10.18170/DVN/WBO7LK

Competing interests:

Authors have no conflict of interests to disclose.

Funding:

The research results of this article are sponsored partly by the Kunshan Municipal Government research funding.

The funder played no role in study design, data collection, and analysis, the decision to publish, or preparation of the manuscript.

Author Contributions:

THZ conceived of and conducted the data analyses, interpreted the findings, and wrote the manuscript. LJY, HSC and HYJ contributed to manuscript revision. CKW contributed to analysis and interpretation of data and drafting and revision of the manuscript.

Acknowledgements:

Not Applicable

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Table 1.

Cut-point for each of nine biomarkers used to construct allostatic load.

Biomarkers	Cut-points	
	Male	**Female**
Body mass index, kg/m^2	≤19.33 kg/m^2	≤17.78 kg/m^2
Glucose, mmol/L	≥5.13 mmol/L	≥5.15 mmol/L
Total cholesterol, mmol/L	≤3.51 mmol/L	≤3.71 mmol/L
High-density lipoprotein cholesterol, mmol/L	≤1.04 mmol/L	≤1.06 mmol/L
Triglyceride, mmol/L	≤0.56 mmol/L	≤0.63 mmol/L
High-sensitive C reactive protein, mg/L	≥2.44 mg/L	≥2.33 mg/L
Heart rate, beats/min	≥80 beats/min	≥83 beats/min
Systolic blood pressure, mmHg	≥150 mmHg	≥160 mmHg
Diastolic blood pressure, mmHg	≥90 mmHg	≥90 mmHg

High-risk group was defined as below the sex-specific 25th percentile for body mass index, total cholesterol, high-density lipoprotein cholesterol and triglyceride. High-risk group was defined as above the sex-specific 75th percentile for glucose, high-sensitive C reactive protein, heart rate, systolic blood pressure, and diastolic blood pressure.

Table 2.
Baseline characteristics by allostatic load burden (low, medium, and high) among males and females.

	Male (N=709)					Female (N=810)				
	Total	Low (n=181)	Medium (n=455)	High (n=73)	P-value	Total	Low (n=231)	Medium (n=534)	High (n=45)	P-value
Age, years, mean±SD	80.5±11.3	77.6±10.7	81.0±11.4	84.1±11.0	0.042	90.2±11.8	87.0±13.0	91.3±11.1	93.6±11.0	0.029
Married, No. (%)	446 (62.9%)	125 (69.1%)	276 (60.7%)	45 (61.6%)	0.137	173 (21.4%)	65 (28.1%)	101 (18.9%)	7 (15.6%)	0.010
Han ethnicity, No. (%)	638 (89.9%)	168 (92.8%)	402 (88.4%)	68 (93.2%)	0.152	719 (88.8%)	210 (90.9%)	471 (88.2%)	38 (84.4%)	0.354
Any education, No. (%)	457 (64.5%)	119 (65.8%)	294 (64.6%)	44 (60.3%)	0.707	108 (13.3%)	50 (21.7%)	55 (10.3%)	3 (6.7%)	0.000
Smoking, No. (%)					0.418					0.800
Current Smoker	265 (37.3%)	71 (39.2%)	170 (37.4%)	24 (32.9%)		30 (3.7%)	6 (2.6%)	23 (4.3%)	1 (2.2%)	
Past Smoker	109 (15.4%)	22 (12.2%)	71 (15.6%)	16 (21.9%)		16 (2.0%)	5 (2.2%)	11 (2.1%)	0	
Non-smoker	335 (47.3%)	88 (48.6%)	214 (47.0%)	33 (45.2%)		761 (94.3%)	219 (95.2%)	498 (93.6%)	44 (97.8%)	
Exercise, No. (%)	183 (26.2%)	55 (30.6%)	110 (24.6%)	18 (25.0%)	0.300	154 (19.3%)	41 (18.1%)	103 (19.5%)	10 (22.2%)	0.782
Hypertension, No. (%)	125 (17.8%)	0	97 (21.6%)	28 (38.9%)	0.000	179 (22.6%)	0	153 (29.3%)	26 (57.8%)	0.000
Diabetes, No. (%)	13 (1.8%)	3 (1.6%)	4 (0.9%)	6 (8.3%)	0.001	12 (1.5%)	0	10 (1.9%)	2 (4.4%)	0.013
Heart disease, No. (%)	42 (5.9%)	10 (5.5%)	27 (6.0%)	5 (6.9%)	0.921	62 (7.8%)	10 (4.5%)	40 (7.6%)	12 (26.7%)	0.000
Stroke, No. (%)	33 (4.7%)	8 (4.4%)	18 (4.0%)	7 (10.0%)	0.105	45 (5.7%)	14 (6.2%)	29 (5.5%)	2 (4.4%)	0.878
Pulmonary, No. (%)	74 (10.5%)	18 (10.0%)	45 (9.9%)	11 (15.3%)	0.374	49 (6.2%)	10 (4.4%)	38 (7.2%)	1 (2.2%)	0.181
Arthritis, No. (%)	96 (13.6%)	18 (10.0%)	64 (14.2%)	14 (19.2%)	0.132	128 (16.0%)	44 (19.2%)	79 (15.0%)	5 (11.1%)	0.224
Cancer, No. (%)	2 (0.3%)	1 (0.6%)	1 (0.2%)	0	0.591	6 (0.8%)	0	5 (1.0%)	1 (2.2%)	0.133

[a]Married vs. widowed, never married, and divorce.

Table 3.
Association between allostatic load and mortality among males and females.

		Males (N = 709)		
	Events per 1,000 PYs (95% CI)	Unadjusted	Demographically adjusted [a]	Fully adjusted [b]
		Hazard ratio (95% CI)		
AL (continuously)		1.75 (1.44, 2.12)	1.51 (1.24, 1.84)	1.51 (1.23, 1.84)
AL category				
Low (0-1)	66.1 (50.7, 86.1)	Ref.	Ref.	Ref.
Medium (2-4)	110.4 (96.3, 126.7)	1.68 (1.24, 2.26)	1.36 (1.00, 1.83)	1.33 (0.98, 1.80)
High (5-9)	201.3 (153.0, 265.0)	3.06 (2.09, 4.48)	2.29 (1.56, 3.37)	2.18 (1.48, 3.22)
		Females (N = 810)		
	Events per 1,000 PYs (95% CI)	Unadjusted	Demographically adjusted [a]	Fully adjusted [b]
		Hazard ratio (95% CI)		
AL (continuously)		1.45 (1.23, 1.72)	1.16 (0.97, 1.37)	1.16 (0.97, 1.38)
AL category				
Low (0-1)	121.1 (100.7, 145.6)	Ref.	Ref.	Ref.
Medium (2-4)	174.8 (156.9, 194.7)	1.44 (1.17, 1.79)	1.14 (0.92, 1.42)	1.11 (0.89, 1.38)
High (5-9)	252.6 (180.5, 353.5)	2.11 (1.44, 3.10)	1.36 (0.93, 2.01)	1.34 (0.91, 1.97)

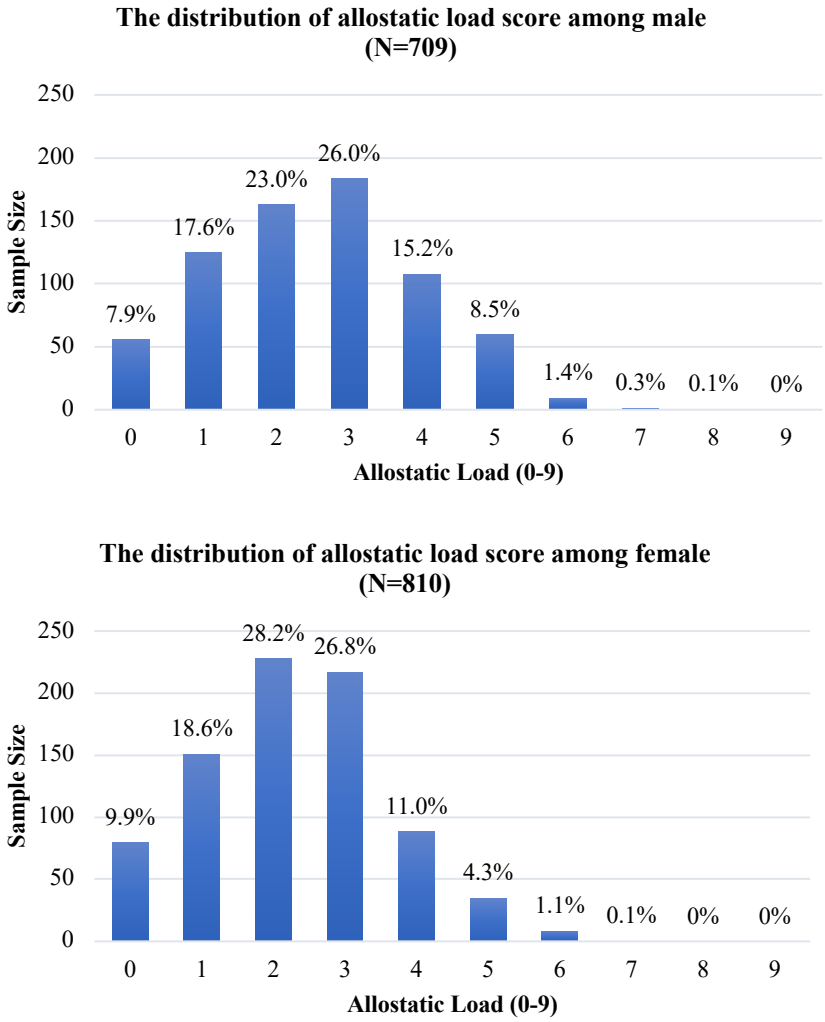
Abbreviations: PY, person-year; CI, confidence interval; AL, allostatic load.

[a]Demographically adjusted model included age, ethnicity (Han vs. minority), education (any vs. none), and marital status (married vs. others).

[b]Fully adjusted model included age, ethnicity (Han vs. minority), education (any vs. none), and marital status (married vs. others), smoking (current vs. previous or non-smoke), exercise (yes vs. no), and chronic diseases such as pulmonary disease and arthritis.

Figure 1. Distribution of sample size by allostatic load score among males and females.

Figure 1.
Distribution of sample size by allostatic load score among males and females.



The above figure is the distribution of allostatic load score among male. The below figure is the distribution of allostatic load score among female. The distribution of the AL score (range: 0-9) is right skewed for both males and females; only 13 (1.8%) males and 10 (1.2%) females had a score of 6-9, respectively.

Table S1. The cut-points for excluding SBP, DBP, BMI, and heart rate.

Biomarkers	Cut-points
Body mass index, kg/m^2	>40
Heart rate, beats/min	>220
Systolic blood pressure, mmHg	>200 or <90
Diastolic blood pressure, mmHg	>110 or <60

Table S2. Baseline characteristics between included and excluded participants.

Characteristics	Included (N=1,519)	Excluded [b] (N=920)	P-value
Age, years, mean±SD	85.6±12.6	85.6± 13.5	0.523
Married [a], No. (%)	619 (40.8%)	361 (39.2%)	0.461
Han ethnicity, No. (%)	1,357 (89.3%)	836 (90.9%)	0.223
Any education, No. (%)	565 (37.2%)	384 (41.7%)	0.026
Smoking, No. (%)			0.460
Current Smoker	295 (19.5%)	170 (18.7%)	
Past Smoker	125 (8.3%)	88 (9.7%)	
Non-smoker	1,096 (72.3%)	650 (71.6%)	
Exercise, No. (%)	337 (22.5%)	271 (30.2%)	0.000
Hypertension, No. (%)	304 (20.3%)	201 (22.8%)	0.153
Diabetes, No. (%)	25 (1.7%)	19 (2.1%)	0.405
Heart disease, No. (%)	104 (6.9%)	48 (5.4%)	0.138
Stroke, No. (%)	78 (5.2%)	62 (6.9%)	0.077
Pulmonary, No. (%)	123 (8.2%)	73 (8.2%)	0.994
Arthritis, No. (%)	224 (14.9%)	152 (17.1%)	0.145
Cancer, No. (%)	8 (0.5%)	3 (0.3%)	0.341

[a]Married vs. widowed, never married, and divorced.

[b]Excluded criteria includes incomplete data on AL biomarkers, no follow-up data, missing data or extreme values of the biomarkers, and aged less than 60 years.

Table S3. Compare participant who lost follow up data and who included in study.

Characteristics	Included (N=1,519)	Loss follow up (N=522)	P-value
Age, years, mean±SD	85.6±12.6	87.3± 13.3	0.98
Married [a], No. (%)	619 (40.8%)	180 (34.8%)	0.02
Han ethnicity, No. (%)	1,357 (89.3%)	467 (90.2%)	0.60
Any education, No. (%)	565 (37.2%)	202 (39.0%)	0.47
Smoking, No. (%)			
Current Smoker	295 (19.5%)	88 (17.3%)	0.54
Past Smoker	125 (8.3%)	45 (8.8%)	
Non-smoker	1,096 (72.3%)	376 (73.9%)	
Exercise, No. (%)	337 (22.5%)	129 (25.6%)	0.15
Hypertension, No. (%)	304 (20.3%)	109 (21.9%)	0.45
Diabetes, No. (%)	25 (1.7%)	7 (1.4%)	0.68
Heart disease, No. (%)	104 (6.9%)	25 (5.0%)	0.13
Stroke, No. (%)	78 (5.2%)	44 (8.8%)	0.004
Pulmonary, No. (%)	123 (8.2%)	39 (7.7%)	0.77
Arthritis, No. (%)	224 (14.9%)	79 (15.8%)	0.62
Cancer, No. (%)	8 (0.5%)	2 (0.4%)	0.72

[a]Married vs. widowed, never married, and divorced.

Table S4. Sensitivity analysis.

		Males (N = 709)		
	Events per 1,000 PYs (95% CI)	Unadjusted	Demographically adjusted [a]	Fully adjusted [b]
		Hazard ratio (95% CI)		
AL (continuously)		1.28 (1.18, 1.38)	1.18 (1.09, 1.28)	1.18 (1.08, 1.27)
AL category				
Low (0-1)	68.1 (53.4, 86.9)	Ref.	Ref.	Ref.
Medium (2-4)	115.4 (100.7, 132.1)	1.70 (1.29, 2.25)	1.45 (1.10, 1.93)	1.41 (1.06, 1.88)
High (5-9)	214.7 (154.8, 297.6)	3.18 (2.11, 4.78)	2.33 (1.54, 3.53)	2.35 (1.55, 3.56)
		Females (N = 810)		
	Events per 1,000 PYs (95% CI)	Unadjusted	Demographically adjusted [a]	Fully adjusted [b]
		Hazard ratio (95% CI)		
AL (continuously)		1.22 (1.14, 1.31)	1.10 (1.02, 1.18)	1.09 (1.01, 1.17)
AL category				
Low (0-1)	113.7 (96.2 134.4)	Ref.	Ref.	Ref.
Medium (2-4)	191.7 (171.8, 213.9)	1.70 (1.39, 2.08)	1.29 (1.05, 1.57)	1.27 (1.03, 1.56)
High (5-9)	267.4 (168.5, 424.5)	2.40 (1.47, 3.93)	1.37 (0.83, 2.25)	1.39 (0.85, 2.29)

Abbreviations: PY, person-year; CI, confidence interval; AL, allostatic load.

[a]Demographically adjusted model included age, ethnicity (Han vs. minority), education (any vs. none), and marital status (married vs. others).

[b]Fully adjusted model included age, ethnicity (Han vs. minority), education (any vs. none), and marital status (married vs. others), smoking (current vs. previous or non-smoke), exercise (yes vs. no), and chronic diseases such as pulmonary disease and arthritis.

zSTROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	1-2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	5-6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up (*b*) For matched studies, give matching criteria and number of exposed and unexposed	6-7
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7-9
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7-9
Bias	9	Describe any efforts to address potential sources of bias	7
Study size	10	Explain how the study size was arrived at	6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7-9
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding (*b*) Describe any methods used to examine subgroups and interactions (*c*) Explain how missing data were addressed (*d*) If applicable, explain how loss to follow-up was addressed (*e*) Describe any sensitivity analyses	9
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (b) Give reasons for non-participation at each stage (c) Consider use of a flow diagram	10
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (b) Indicate number of participants with missing data for each variable of interest (c) Summarise follow-up time (eg, average and total amount)	10
Outcome data	15*	Report numbers of outcome events or summary measures over time	11-12

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (*b*) Report category boundaries when continuous variables were categorized (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	11-12
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	11-12
Discussion			
Key results	18	Summarise key results with reference to study objectives	12
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	15
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12-14
Generalisability	21	Discuss the generalisability (external validity) of the study results	15
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	17-18

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.