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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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3 **The Association between Allostatic Load and Mortality among Chinese Older Adults:**
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5 **The Chinese Longitudinal Health and Longevity Study**
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10 Tianhang Zhang (MSc.)^{1,2}, Lijing L. Yan (PhD, Prof.)^{1,3}, Huashuai Chen (PhD, Dr.)⁴, Hai-Yu
11
12 Jin (MA)¹, Chenkai Wu (PhD, Prof, Assistant.)^{1,3}
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14
15
16

17 ¹ Global Health Research Center, Duke Kunshan University, Jiangsu, China, ²Imperial
18 College London, United Kingdom, ³ Duke Global Health Institute, Duke University, Durham,
19 North Carolina, ⁴ Center for the Study of Aging and Human Development and the Geriatric
20
21 Division of School of Medicine, Duke University, Durham, North Carolina
22
23
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25
26
27
28
29

30 **Corresponding Author:**

31
32 Chenkai Wu, PhD, MPH, MS

33
34 Global Health Research Center

35
36 Duke Kunshan University

37
38 Academic Building 3038

39
40 No. 8 Duke Avenue, Kunshan, Jiangsu, China, 215316

41
42 Phone: (+86) 512 36657235

43
44 Email: chenkai.wu@dukekunshan.edu.cn
45
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3 **The Association between Allostatic Load and Mortality among Chinese Older Adults:**
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5 **The Chinese Longitudinal Health and Longevity Study**
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8 **Abstract**
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10 **Background:** Allostatic load has shown that high burden of AL is associated with increased
11 risk of adverse outcomes, but little attention has been paid to China with largest aging
12 population in the world.
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19 **Objective:** This study is to examine the association between allostatic load (AL) and all-cause
20 mortality among Chinese adults aged at least 60 years.
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26 **Design:** Population-based prospective cohort study.
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31 **Setting:** In 2011-2012, an ancillary study, in which a blood test was added, including a total of
32 2,439 participants, was conducted in eight longevity areas in the Chinese Longitudinal Healthy
33 Longevity Survey.
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40 **Participants:** The final analytic sample consisted of 1,519 participants (mean \pm SD age: men
41 80.5 \pm 11.3 years; women 90.2 \pm 11.8 years; and 53% women).
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47 **Primary outcome measure:** Cox models were used to examine the association between AL
48 and mortality among men and women, separately. Analysis were also adjusted for potential
49 confounders including age, ethnicity, education, and marital status, smoking and exercise.
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3 **Results:** Male with a medium AL burden (score: 2-4) and high AL burden (score: 5-9) had a
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5 33% and 118% higher hazard of death, respectively, than those with a low AL burden (score:
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7 0-1). We did not find significant difference between females with different levels of AL burden.
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12 **Conclusion:** Higher AL burden was associated with increased all-cause mortality among
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14 Chinese men aged at least 60 years. However, we did not find strong association among women.
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16 In conclusion, Intervention programs targeting modifiable components of the AL burden may
17
18 help prolong lifespan for older adults, especially men, in China.
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24 **Keywords:** Allostatic load; Mortality; China; Older adults.
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27 ARTICLE SUMMARY

28 **Article focus**

- 29 • Is a higher burden of AL associated with increased risk of all-cause mortality among
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31 both older men and women in China?
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36 **Key messages**

- 37 • Higher AL burden was associated with increased all-cause mortality among Chinese
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39 men aged at least 60 years.
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- 43 • We did not find strong evidence about Allostatic load was associated with specific
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45 causes of death over the same follow-up period among women.
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48 **Strengths and limitations of this study**

- 49 • This is the first to investigate the association between AL and mortality using a Chinese
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51 population.
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- 54 • The CLHLS dataset that is a large nationally representative old population survey in
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56 China.
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- 59 • The updated quartile risk method for biomarkers BMI, total cholesterol, and triglyceride
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3 among older adults.
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- 5 • We did not include any primary neuroendocrine biomarkers such as cortisol in
6 constructing the AL score.
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- 8 • There is huge loss to follow up (>20%; 552 of 2439), although only 3 to 7-year range
9 of follow up (2011 to 2014-18), which may underestimate the association.
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3 **The Association between Allostatic Load and Mortality among Chinese Older Adults:**
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5 **The Chinese Longitudinal Health and Longevity Study**
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10 BACKGROUND
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12 Allostatic load (AL) is conceptualized as the cumulative wear and tear on multiple
13 physiological systems resulting from repeated adaptation to stressors [17,23,24]. In the absence
14 of a gold standard, many operational definitions of AL have been proposed. The most
15 commonly used construct of AL was developed by Seeman and colleagues who have used two
16 categories of biomarkers for quantifying AL [15,24]. The first category (called primary
17 mediators) includes biomarkers the body releases in response to stress, such as cortisol and
18 dehydroepiandrosterone sulphate (DHEA-S); the second category comprises comprises
19 secondary outcomes that result from the effects of primary mediators. The examples of
20 biomarkers are blood pressure (BP), cholesterol, and waist-hip ratio [24].
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35 A number of studies identified that a high burden of AL is associated with increased risk of
36 adverse outcomes including cardiovascular disease, functional decline, and mortality among
37 older adults [1,13,14,17,18,20,25]. For example, in a 7-year longitudinal study conducted in
38 2006, increased AL score was associated with higher mortality among older population [18].
39 In a cohort study of 1,023 community-dwelling older adults in Taiwan, researchers found that
40 higher AL score was related with higher death rate [14]. Additionally, some studies found that
41 women and men experienced chronic stress in different ways. For example, Yang et al in 2011
42 revealed gender difference in the AL biomarkers and the age trajectories of physiological
43 dysregulation [33]. Women had higher level of inflammation biomarkers but lower risk of
44 cardiovascular disease (CVD) than men. Another study from Tampubolon and Maharani in
45 2018 among older population found that AL score increased in sex difference [34]. Compared
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3 with men, women showed advantage in life expectancy [34]. Taken together, these results
4 suggest the use of sex-specific cut-off points for AL biomarkers in future research.
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10 A number of studies have examined the association between AL and mortality among older
11 adults. However, little attention has been paid to less developed regions, including China – the
12 most populous country with the largest aging population in the world. In 2019, there were 249
13 million adults aged 60 years or above in China, accounting for 17.3% of its total population,
14 and this number is projected to almost double in 2050, reaching 487 million [4,5].
15 Understanding the relationship between AL and mortality in less developed country is
16 beneficial for leading to interventions, which could be helpful to change unhealthy lifestyle,
17 decrease morbidity and mortality among older population. In addition, less studies focus on
18 sex-specific cut off points for calculating AL index, this study will conduct sex-specific studies,
19 which may closely reflect AL score among older population.
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35 In this study, we used a large cohort study to examine the association between AL and all-
36 causes mortality among Chinese men and women aged at least 60 years. We hypothesized that
37 a higher burden of AL would be associated with increased risk of all-cause mortality among
38 both older men and women in China.
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47 METHODS

48 **Data and Study Participants**

49 We used data from the Chinese Longitudinal Healthy Longevity Survey (CLHLS), an ongoing
50 prospective, longitudinal study with the largest sample of the oldest old in China. Half of the
51 counties and cities in 22 of the 31 provinces in China (covering 85% of the population) were
52 randomly selected through a multistage cluster sampling approach. A wide range of socio-
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3 demographic, lifestyle, and health measures were collected in the CLHLS. The baseline survey
4 was conducted in 1998 and participants who were alive were re-interviewed in each follow-up
5 survey (2000, 2002, 2005, 2008-2009, 2011-2012, 2014, and 2017-2018). In 2011-2012, an
6 ancillary study, in which a blood test was added, was conducted in eight longevity areas:
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8 Laizhou City in Shandong Province, Xiayi County in Henan Province, Zhongxiang City in
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10 Hubei Province, Mayang County in Hunan Province, Yongfu County in Guangxi Autonomous
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12 Area, Sanshui District in Guangdong Province, Chengmai County in Hainan Province, and
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14 Rudong County in Jiangsu Province. The Research Ethics Committees of Peking University
15
16 and Duke University granted approval for the Protection of Human Subjects for the CLHLS.
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18 All study participants gave informed consent. A more detailed description of the recruitment
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20 strategy and study design of the CLHLS has been published elsewhere [11,31,32]. A total of
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22 8,959 individuals were included at baseline (1998). 1998 baseline survey, which was extended
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24 to 11,162 in 2000, it was found that almost 30 percent died before 2002 interview and
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26 approximately 14 percent were lost that was higher than the attrition rate between 1998 and
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28 2000 wave (9.6 percent); the number of participants were extend to 16,064 in 2002, and about
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30 13.8 percent were lost between 2002 and 2005; the number of interviewed were 15,638 in 2005,
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32 and about 13.2 percent were lost between 2005 and 2008-2009; the number of participants were
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34 extended to 16,540, and approximately 17.7 percent were lost between 2008-2009 and 2011-
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36 2012; the total number of interviewed participants were 9765 in 2011-2012 [35].
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49 A total of 2,439 persons contributed blood sample in the ancillary study (2011-2012).
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51 Participants were excluded from the analytic sample if they had (i) incomplete data on any
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53 biomarkers for constructing AL (n = 251), (ii) no follow-up data (time to death or censorship
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55 was undetermined; n = 552), (iii) had extreme values on the biomarkers (n = 109), or (iv) were
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57 less than 60 years old (n=16). The final analytic sample consisted of 1,519 participants. We did
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3 not observe appreciable differences in age, ethnicity, marital status, smoking, or chronic
4 conditions between the analytic sample and those excluded (n = 920; Table S1). Compared to
5 the analytic sample, excluded persons had higher education level and higher prevalence of
6 exercise. In addition, compared to people who were loss follow up, the included people had
7 higher prevalence of married, and higher prevalence of stroke; we did not observe appreciable
8 differences in age, ethnicity, smoking, exercise, hypertension, diabetes, heart disease,
9 pulmonary, arthritis, and cancer between both (Table S4).

21 **Calculation of AL Score**

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

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49 To be in line with previous studies [6,8,24,25], we used the highest quartile for heart rate, SBP,
50 DBP, glucose, and CRP and the lowest quartile for HDL cholesterol to define high-risk group
51 (coded 1). Because BMI, total cholesterol, and triglyceride were inversely associated with
52 mortality among older adults, especially the oldest old [19,29,30], we used the lowest quartile
53 to define high-risk group for these three biomarkers. For participants who self-reported having
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3 been diagnosed with hypertension and heart disease, we classified their SBP, DBP, and glucose
4 into the high-risk category. Similarly, we classified participants' glucose into the high-risk
5 group if they self-reported having been diagnosed with diabetes. The identification of risk
6 quartiles of biomarkers is commonly used to construct AL index [15, 36]. The cut-points of all
7 nine AL components by men and women were presented in Table 1. We constructed the AL
8 score based on the count of biomarkers falling in the high-risk group, ranging from 0 (lowest)
9 to 9 (highest). To be in line with previous studies [37], we then considered using similar cut-
10 off points, classifying the AL score into three categories based on sample distribution: 0-1 (low
11 burden), 2-4 (medium burden), and 5-9 (high burden).
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26 **Mortality**

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

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44 Demographic and lifestyle characteristics were collected by interview, including age, sex,
45 ethnicity, education, and marital status, smoking status, and physical exercise. We divided
46 ethnicity into Han and others (minority groups). Years of education were dichotomized as any
47 (one year or more) and no education, which is commonly way used in CLHLS study [38, 39].
48 Marital status was dichotomized as married and others (widowed, not married, and divorced).
49 Cigarette smoking was categorized as current, past, and non-smoker. Information of exercise
50 was collected using the question "Do you do exercise at present?" and dichotomized into yes
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3 or no. Chronic conditions were measured based on self-reported physician's diagnosis,
4 including hypertension, diabetes, heart disease, stroke, pulmonary disease (including bronchitis,
5 emphysema, pneumonia and asthma), arthritis, and cancer.
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10 11 12 **Statistical Analyses**

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14 All analyses were conducted separately for males and females. We first presented the relative
15 frequency of the AL score using histograms and calculated mean AL score. Then, we described
16 the baseline characteristics of study sample by AL burden (low, medium, and high) using
17 means and SDs for continuous variables and counts and percentages for categorical variables.
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19 Characteristics were compared across the three AL categories using analyses of variance for
20 continuous variables and chi-square tests for categorical variables.
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31 We calculated the death rates across three AL categories (low, medium, and high burden). We
32 used the Cox proportional hazards model to determine the unadjusted and adjusted associations
33 between the AL and all-cause mortality. Age, sex, education, and marital status were included
34 in the demographically adjusted models; smoking status, physical exercise, and chronic status
35 including pulmonary disease and arthritis were added in the fully adjusted models. We
36 modelled AL both continuously and in categories.
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47 Furthermore, one sensitivity analysis was undertaken, which aimed to exam if the model results
48 were influenced when we did not use self-reported hypertension or diabetes to classify
49 participants' risk category for BP or glucose.
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56 All tests were two-sided with a significance level of P-value less than 0.05. We conducted all
57 analyses using STATA version 16.0 (Stata Corp, College Station, TX).
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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

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

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17 A total of 310 males died; the overall death rate was 105.7 per 1,000 person-years. Males with
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19 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
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21 201.3 per 1,000 person-years, respectively (Table 3).
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27 In the unadjusted Cox model, per unit higher AL score was significantly associated with a 75%
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29 higher hazard of death among males (95% CI: 44%, 112%; Table 3). The association slightly
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31 attenuated but persisted in the full adjusted model (hazard ratio [HR] = 1.51, 95% CI: 1.23,
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33 1.84). When modelled in categories, in the unadjusted model, the hazard of death of male with
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35 the medium AL burden (score: 2-4) was 1.68 times than hazard of death of those with a lower
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37 AL burden (score: 0-1); Male with a high AL burden (Score: 5-9) had a more than three-fold
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39 hazard of death than those with a lower AL burden (score: 0-1). These associations persisted
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41 after adjustment of socio-demographics and lifestyles. In the fully adjusted model, males with
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43 a medium AL burden (score: 2-4) had a 33% higher hazard of death than those with a low AL
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45 burden (score: 0-1). Males with a high AL burden (score: 5-9) had a more than two-fold hazard
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47 of death than those with a low AL burden (score: 0-1). Results did not change substantially in
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49 the sensitivity analyses (Tables S3).
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56 **Association between Allostatic Load and Mortality among Females**

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3 Over an average follow-up period of 3.9 years, 787 deaths (51.8%) occurred. A total of 477
4 females died (58.9%); the overall death rate was 161.3 per 1,000 person-years. The death rates
5 for females with an AL score of 0-1 (low burden), 2-4 (medium burden), and 5-9 (high burden)
6 were 121.1, 174.8, and 252.6 per 1,000 person-years, respectively (Table 3).
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14 In the unadjusted Cox model, per unit higher AL (modelled continuously) was significantly
15 associated with a 45% higher hazard of death among females (95% CI: 23%, 72%; Table 3).
16 However, the association attenuated and became insignificant after adjusting for socio-
17 demographics (age, sex, ethnicity, education, and marital status); similar results were observed
18 when smoking, exercise, and chronic conditions including pulmonary disease and arthritis were
19 additionally adjusted (HR = 1.16, 95% CI: 0.97, 1.38). When modelled in categories, the HR
20 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
21 2-4 and 5-9, respectively, compared with those with a score of 0-1 in the unadjusted model.
22 After multivariable adjustment, females with an AL score of 2-4 and 5-9 had a 11% and 34%
23 higher hazard of death, although the associations were not significant. Results did not change
24 substantially in the sensitivity analyses (Tables S3).
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43 DISCUSSION

44 The present study aimed to explore the association between AL burden and all-cause mortality
45 among men and women aged at least 60 years in China. We found that older men with high
46 AL burden had a more than two-fold hazard of death than those with a low AL burden.
47 However, the association was less clear among women. These findings were in line with
48 previous studies showing men tend to have higher AL with higher risk of death than women,
49 and gender difference among AL score and cause-specific mortality risk including infectious
50 diseases, cardiometabolic disease, and malignant neoplasm [14,26,27]. One possible
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3 explanation for the sex difference in the association between AL and mortality among older
4 adults is that older women might be less vulnerable to stress men due to sex difference in
5 hippocampal formation in humans [10,16,21]. In addition, it has been shown that estrogen plays
6 an important role in brain with the development of aging, aiming to maintain allostasis when
7 facing physiological stress, which may be possible to protect women against age-related
8 diseases [3,22,28]. Moreover, Gruenewald et al. (2006) stated that gender difference in
9 forecasting mortality risk among older people by biomarkers; compared with female,
10 neuroendocrine and immune related biomarkers were more predictive in male [12]. In addition,
11 the sex difference in the association between AL and mortality may be explained by behavioral
12 factors. Social support is an effective in relieving stress [40]. Women are more socially active
13 to seek emotional support when facing stress than men [40]. Furthermore, in our study, women
14 had high mean age, less education, lower prevalence of smoking, and less exercise. Therefore,
15 adjustment of these covariates may influence the significant level of association in final model.
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35 Previous study has identified increased risk of all-cause mortality associated with increasing
36 AL score in men [14]. We found that the strength of the association differed between the
37 present study and Hwang et al.'s work. There are several plausible explanations for this
38 discrepancy. First, the population in Hwang et al.'s study (≥ 54 years) was younger than ours
39 (≥ 60 years). Second, we did not include cortisol, which is a commonly used indicator of the
40 primary mediator stress, due to data unavailability. Surrogate measures were used in the present
41 study, which may lead to weaker associations. Third, two studies used different cut-points for
42 constructing the AL score. We used sex-specific cut-off points for each biomarker; while
43 general cut-points were used in Hwang et al.'s work. Fourth, follow-up length was different
44 between the two studies.
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3 For the sensitivity analysis of association between AL and mortality, the overall magnitude of
4 the HR was not largely altered, and the association were still statically significant. This suggest
5 that the results of association between AL category and mortality may not influenced by the
6 participants who were self-reported disease status, which may support that our results are
7 robust (Table S3).
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12 The AL was initially constructed using primary mediator stress including cortisol, epinephrine,
13 and norepinephrine. These biomarkers are not widely available and secondary responses of
14 cardiovascular, inflammatory and metabolic biomarkers such as CRP, BP and heart rate were
15 used as surrogate measures. Although multiple CVD risk factors were included, AL, in theory,
16 represents multisystem physiological dysregulation instead of functional decline in one system.
17 Previous studies showed that AL was able to stratify the risk of a wider range health outcomes
18 than traditional CVD risk factors [24], it appears that AL could predict more health information
19 including CVD incident, decline of cognition function, decline of physical function, and
20 mortality than traditional CVD risk factors. The current study did not have data on primary
21 mediator of AL, so we did not conduct separate analysis to investigate the association between
22 primary vs. secondary mediators of AL and mortality.
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42 This study has some strengths. This is among the first to investigate the association between
43 AL and mortality using a Chinese population. Moreover, our study used of CLHLS dataset that
44 is a large nationally representative old population survey in China. Furthermore, we updated
45 quartile risk method for biomarkers BMI, total cholesterol, and triglyceride due to inversely
46 association with mortality among older adults, which may more truly reflect AL score in old
47 people. Additionally, our study added evidence to support sex difference in the association
48 between AL and the increased risk of mortality. Finally, sex-specific cut-off points were used
49 to construct the AL score may more truly reflect association between AL and mortality in
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3 different gender compared previous study. Additionally, we collected detailed covariates
4 information including age, sex, marital status, ethnicity, education, chronic diseases, which
5 enable us to adjust for a range of potential confounders in the final cox model.
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12 Despite these strengths, we acknowledge some limitations. First, we did not include any
13 primary neuroendocrine biomarkers such as cortisol in constructing the AL score due to data
14 limitation. The cortisol biomarker plays an important role in responding stress, which needs
15 repeated measurements within 1-2 days which causes difficulties to measure in large national
16 survey [7]. Inclusion of cortisol biomarker maybe improve our power of AL score predictions
17 for mortality. Additionally, we classified AL biomarkers based on sex-specific quartiles;
18 however, these measures may vary over time, leading to misclassification. Furthermore, there
19 is huge loss to follow up (>20%; 552 of 2439), although only 3 to 7-year range of follow up
20 (2011 to 2014-18), which may underestimate the association. Lastly, participants who provided
21 blood sample in this study were residents in eight longevity areas including Laizhou of
22 Shandong Province, Xiayi of Henan Province, Zhongxiang of Hubei Province, Mayang of
23 Hunan Province, Sanshui of Guangdong Province, Yongfu of Guangxi Autonomous Region,
24 Chengmai of Hainan Province, Rudong of Jiangsu Province, which were from 8 of 23
25 provinces, five autonomous regions, four municipalities, and two special administrative
26 regions. Therefore, our results may not be greatly generalizable to older adults living in other
27 regions of China.
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51 Therefore, even though we found that higher AL burden was associated with increased all-
52 cause mortality among Chinese men aged at least 60 years, but not women, it is really
53 recommended that these results need to be replicated in large longitudinal studies with more
54 longer follow-up time, with more AL biomarkers such as cortisol, or with containing Chinese
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3 from more regions apart from eight longevity areas. This would helpful for comparing with our
4 results and validating them in different population.
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10 CONCLUSION

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

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24 THZ conceived of and conducted the data analyses, interpreted the findings, and wrote the
25
26 manuscript. LJY, HSC and HYJ contributed to manuscript revision. CKW contributed to
27
28 analysis and interpretation of data and drafting and revision of the manuscript.

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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	≤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

^aMarried vs. widowed, never married, and divorce.

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

Males (N = 709)				
		Unadjusted	Demographically adjusted ^a	Fully adjusted ^b
Events per 1,000 PYs (95% CI)		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)				
		Unadjusted	Demographically adjusted ^a	Fully adjusted ^b
Events per 1,000 PYs (95% CI)		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.

^aDemographically adjusted model included age, ethnicity (Han vs. minority), education (any vs. none), and marital status (married vs. others).

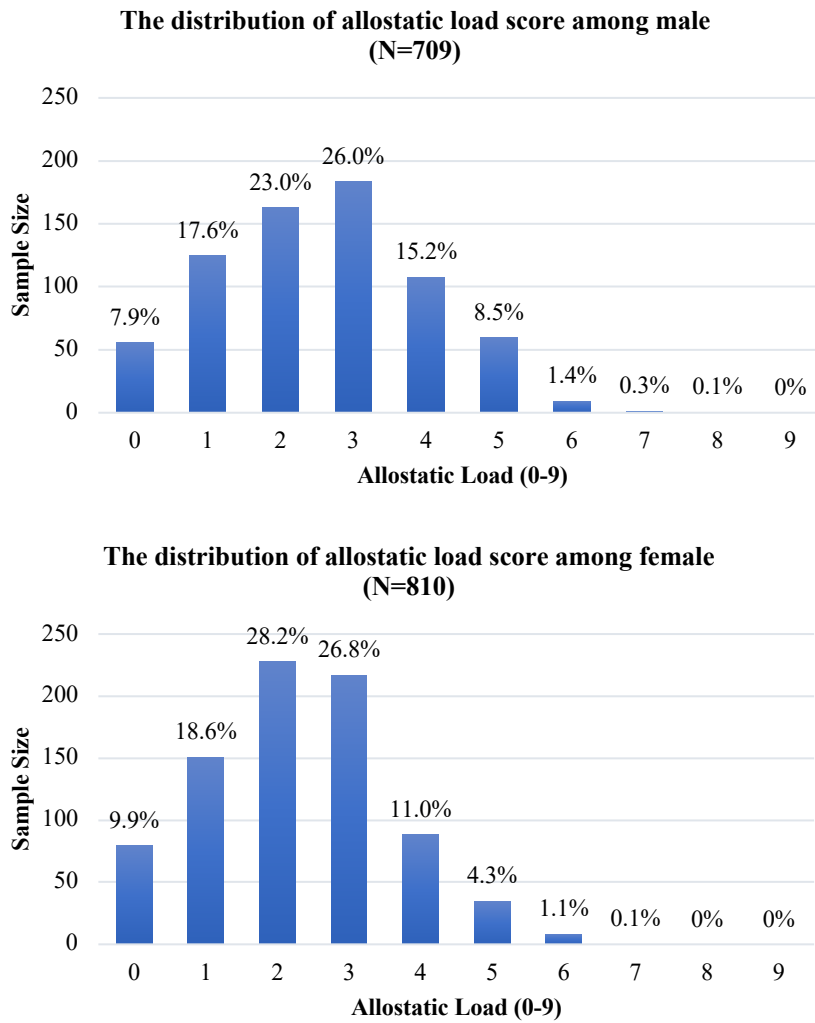
^bFully adjusted model included age, ethnicity (Han vs. minority), education (any vs. none), and marital status (married vs. others), smoking (current vs. previous or non-smoke), exercise (yes vs. no), and chronic diseases such as pulmonary disease and arthritis.

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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. (%)			
Current Smoker	295 (19.5%)	170 (18.7%)	0.460
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

^aMarried vs. widowed, never married, and divorced.^bExcluded 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.

^aDemographically adjusted model included age, ethnicity (Han vs. minority), education (any vs. none), and marital status (married vs. others).

^bFully 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

^aMarried 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

1	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	11-12
2			(b) Report category boundaries when continuous variables were categorized	
3			(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
4				
5				
6				
7				
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9	Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	11-12
10				
11	Discussion			
12				
13	Key results	18	Summarise key results with reference to study objectives	12
14	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
15				
16	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
17				
18				
19	Generalisability	21	Discuss the generalisability (external validity) of the study results	15
20				
21	Other information			
22	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
23				
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*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>.

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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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3 **The Association between Allostatic Load and Mortality among Chinese Older Adults:**
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5 **The Chinese Longitudinal Health and Longevity Study**
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10 Tianhang Zhang (MSc.)^{1,2}, Lijing L. Yan (PhD, Prof.)¹, Huashuai Chen (PhD, Dr.)³, Hai-Yu
11
12 Jin (MA)¹, Chenkai Wu (PhD, Prof, Assistant.)¹
13
14
15
16

17 ¹ Global Health Research Center, Duke Kunshan University, Jiangsu, China, ²Imperial
18
19 College London, United Kingdom, ³ Center for the Study of Aging and Human Development
20
21 and the Geriatric Division of School of Medicine, Duke University, Durham, North Carolina
22
23
24
25
26
27

28 **Corresponding Author:**
29

30 Chenkai Wu, PhD, MPH, MS
31

32 Global Health Research Center
33

34 Duke Kunshan University
35

36 Academic Building 3038
37

38 No. 8 Duke Avenue, Kunshan, Jiangsu, China, 215316
39

40 Phone: (+86) 512 36657235
41

42 Email: chenkai.wu@dukekunshan.edu.cn
43
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3 **The Association between Allostatic Load and Mortality among Chinese Older Adults:**
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5 **The Chinese Longitudinal Health and Longevity Study**
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7

8 **Abstract**
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10 **Background:** Allostatic load has shown that high burden of AL is associated with increased
11 risk of adverse outcomes, but little attention has been paid to China with largest aging
12 population in the world.
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19 **Objective:** This study is to examine the association between allostatic load (AL) and all-cause
20 mortality among Chinese adults aged at least 60 years.
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26 **Design:** Population-based prospective cohort study.
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31 **Setting:** In 2011-2012, an ancillary study, in which a blood test was added, including a total of
32 2,439 participants, was conducted in eight longevity areas in the Chinese Longitudinal Healthy
33 Longevity Survey.
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40 **Participants:** The final analytic sample consisted of 1,519 participants (mean \pm SD age: men
41 80.5 \pm 11.3 years; women 90.2 \pm 11.8 years; and 53% women).
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47 **Primary outcome measure:** Cox models were used to examine the association between AL
48 and mortality among men and women, separately. Analysis were also adjusted for potential
49 confounders including age, ethnicity, education, and marital status, smoking and exercise.
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3 **Results:** Male with a medium AL burden (score: 2-4) and high AL burden (score: 5-9) had a
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5 33% and 118% higher hazard of death, respectively, than those with a low AL burden (score:
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7 0-1). We did not find significant difference between females with different levels of AL burden.
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12 **Conclusion:** Higher AL burden was associated with increased all-cause mortality among
13
14 Chinese men aged at least 60 years. However, we did not find strong association among women.
15
16 In conclusion, Intervention programs targeting modifiable components of the AL burden may
17
18 help prolong lifespan for older adults, especially men, in China.
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24 **Keywords:** Allostatic load; Mortality; Chinese; Elderly; Cohort study; disease burden.
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27 ARTICLE SUMMARY

28 **Strengths and limitations of this study**

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- 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.

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3 1 **The Association between Allostatic Load and Mortality among Chinese Older Adults:**
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5 2 **The Chinese Longitudinal Health and Longevity Study**
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10 4 BACKGROUND
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12 5 Allostatic load (AL) is conceptualized as the cumulative wear and tear on multiple
13
14 6 physiological systems resulting from repeated adaptation to stressors [1,2,3,4]. In the absence
15
16 7 of a gold standard, many operational definitions of AL have been proposed. The most
17
18 8 commonly used construct of AL was developed by Seeman and colleagues who have used two
19
20 9 categories of biomarkers for quantifying AL [4,5]. The first category (called primary mediators)
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22 10 includes biomarkers the body releases in response to stress, such as cortisol and
23
24 11 dehydroepiandrosterone sulphate (DHEA-S); the second category comprises secondary
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26 12 outcomes that result from the effects of primary mediators. Examples of biomarkers are blood
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28 13 pressure (BP), cholesterol, and the waist-hip ratio [4].
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35 15 A number of studies identified that a high burden of AL is associated with increased risk of
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37 16 adverse outcomes including cardiovascular disease, functional decline, and mortality among
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39 17 the older adults [1,2,6,7,8,9,10]. For example, in a 7-year longitudinal study conducted in 2006,
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41 18 increased AL score was associated with higher mortality among older population [8]. In a
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43 19 cohort study of 1,023 community-dwelling older adults in Taiwan, researchers found that
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45 20 higher AL score was related with higher death rate [7]. Additionally, some studies found that
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47 21 women and men experienced chronic stress in different ways. For example, Yang et al in 2011
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49 22 revealed gender differences in the AL biomarkers and the age trajectories of physiological
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51 23 dysregulation [11]. Women had a higher level of inflammation biomarkers but lower risk of
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53 24 cardiovascular disease (CVD) than men. Another study from Tampubolon and Maharani in
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55 25 2018 among the older population found that AL score increased in sex difference [12].
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3 26 Compared with men, women showed an advantage in life expectancy [12]. Taken together,
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5 27 these results suggest the use of sex-specific cut-off points for AL biomarkers in future research.
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10 29 A number of studies have examined the association between AL and mortality among older
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12 30 adults. However, little attention has been paid to less developed regions, including China – the
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14 31 most populous country with the largest aging population in the world. In 2019, there were 249
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16 32 million adults aged 60 years or above in China, accounting for 17.3% of its total population,
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18 33 and this number is projected to almost double in 2050, reaching 487 million [13,14].
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20 34 Understanding the relationship between AL and mortality in less developed countries is
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22 35 beneficial for leading to interventions, which could be helpful to change unhealthy lifestyles,
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24 36 decrease morbidity and mortality among the older population. In addition, less studies focus
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26 37 on sex-specific cut off points for calculating AL index, this study will conduct sex-specific
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28 38 studies, which may closely reflect AL score among the older population.
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35 40 In this study, we used a large cohort study to examine the association between AL and all-
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37 41 causes mortality among Chinese men and women aged at least 60 years. We hypothesized that
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39 42 a higher burden of AL would be associated with increased risk of all-cause mortality among
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41 43 both older men and women in China.
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47 45 METHODS

48 46 **Data and Study Participants**

49 47 We used data from the Chinese Longitudinal Healthy Longevity Survey (CLHLS), an ongoing
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51 48 prospective, longitudinal study with the largest sample of the oldest old in China. Half of the
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53 49 counties and cities in 22 of the 31 provinces in China (covering 85% of the population) were
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55 50 randomly selected through a multistage cluster sampling approach. A wide range of socio-
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3 51 demographic, lifestyle, and health measures were collected in the CLHLS. The baseline survey
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5 52 was conducted in 1998 and participants who were alive were re-interviewed in each follow-up
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7 53 survey (2000, 2002, 2005, 2008-2009, 2011-2012, 2014, and 2017-2018). In 2011-2012, an
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9 54 ancillary study, in which a blood test was added, was conducted in eight longevity areas:
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11 55 Laizhou City in Shandong Province, Xiayi County in Henan Province, Zhongxiang City in
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13 56 Hubei Province, Mayang County in Hunan Province, Yongfu County in Guangxi Autonomous
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15 57 Area, Sanshui District in Guangdong Province, Chengmai County in Hainan Province, and
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17 58 Rudong County in Jiangsu Province. The Research Ethics Committees of Peking University
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19 59 and Duke University granted approval for the Protection of Human Subjects for the CLHLS.
20
21 60 All study participants gave informed consent. A more detailed description of the recruitment
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23 61 strategy and study design of the CLHLS has been published elsewhere [15,16,17]. A total of
24
25 62 8,959 individuals were included at baseline (1998). 1998 baseline survey, which was extended
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27 63 to 11,162 in 2000, it was found that almost 30 percent died before 2002 interview and
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29 64 approximately 14 percent were lost that was higher than the attrition rate between 1998 and
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31 65 2000 wave (9.6 percent); the number of participants were extend to 16,064 in 2002, and about
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33 66 13.8 percent were lost between 2002 and 2005; the number of interviewed were 15,638 in 2005,
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35 67 and about 13.2 percent were lost between 2005 and 2008-2009; the number of participants were
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37 68 extended to 16,540, and approximately 17.7 percent were lost between 2008-2009 and 2011-
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39 69 2012; the total number of interviewed participants were 9765 in 2011-2012 [18].
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49 71 A total of 2,439 persons contributed blood samples in the ancillary study (2011-2012).
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51 72 Participants were excluded from the analytic sample if they had (i) incomplete data on any
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53 73 biomarkers for constructing AL (n = 251), (ii) no follow-up data (time to death or censorship
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55 74 was undetermined; n = 552), (iii) had extreme values on the biomarkers (n = 109; Table S1),
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57 75 or (iv) were less than 60 years old (n=16). The final analytic sample consisted of 1,519
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3 76 participants. We did not observe appreciable differences in age, ethnicity, marital status,
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5 77 smoking, or chronic conditions between the analytic sample and those excluded (n = 920; Table
6
7 78 S2). Compared to the analytic sample, excluded persons had a higher education level and
8
9 79 higher prevalence of exercise. In addition, compared to people who were lost follow up, the
10
11 80 included people had higher prevalence of married, and higher prevalence of stroke; we did not
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13 81 observe appreciable differences in age, ethnicity, smoking, exercise, hypertension, diabetes,
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15 82 heart disease, pulmonary, arthritis, and cancer between both (Table S3).
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23 84 **Calculation of AL Score**

24 85 Based on previous research [3,7,19,20] and availability of data in the CLHLS, we selected nine
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26 86 biomarkers to construct AL: heart rate, systolic BP (SBP), and diastolic BP (DBP), body mass
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28 87 index (BMI), total cholesterol, high-density lipoprotein (HDL) cholesterol, glucose,
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30 88 triglyceride, and C-reactive protein (CRP). BMI, heart rate, SBP, and DBP were collected from
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32 89 physical examinations. BMI was calculated as body weight (kilograms) divided by height
33
34 90 (meters) squared. SBP and DBP were measured by a mercury sphygmomanometer with an
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36 91 appropriately sized cuff, taken in the seated position after 5 minutes of quiet rest under the
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38 92 supervision of trained research assistants. We used the average of two measurements for further
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40 93 analyses. Blood samples were used for assays of the level of the total cholesterol, HDL
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42 94 cholesterol, glucose, triglyceride, and CRP.
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96 To be in line with previous studies [4,10,21,22], we used the highest quartile for heart rate,
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98 SBP, DBP, glucose, and CRP and the lowest quartile for HDL cholesterol to define the high-
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100 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-

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2
3 101 reported having been diagnosed with hypertension and heart disease, we classified their SBP,
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5 102 DBP, and glucose into the high-risk category. Similarly, we classified participants' glucose
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7 103 into the high-risk group if they self-reported having been diagnosed with diabetes. The
8
9 104 identification of risk quartiles of biomarkers is commonly used to construct AL index [5, 26].
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11 105 The cut-points of all nine AL components by men and women were presented in Table 1. We
12
13 106 constructed the AL score based on the count of biomarkers falling in the high-risk group,
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15 107 ranging from 0 (lowest) to 9 (highest). To be in line with previous studies [27], we then
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17 108 considered using similar cut-off points, classifying the AL score into three categories based on
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19 109 sample distribution: 0-1 (low burden), 2-4 (medium burden), and 5-9 (high burden).
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111 **Mortality**

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

117

118 **Covariates**

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

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3 126 or no. Chronic conditions were measured based on self-reported physician's diagnosis,
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5 127 including hypertension, diabetes, heart disease, stroke, pulmonary disease (including bronchitis,
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7 128 emphysema, pneumonia and asthma), arthritis, and cancer.
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11 12 130 **Statistical Analyses**

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15 131 All analyses were conducted separately for males and females. We first presented the relative
16
17 132 frequency of the AL score using histograms and calculated the mean AL score. Then, we
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19 133 described the baseline characteristics of the study sample by AL burden (low, medium, and
20
21 134 high) using means and SDs for continuous variables and counts and percentages for categorical
22
23 135 variables. Characteristics were compared across the three AL categories using analyses of
24
25 136 variance for continuous variables and chi-square tests for categorical variables.
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30 137

31 138 We calculated the death rates across three AL categories (low, medium, and high burden). We
32
33 139 used the Cox proportional hazards model to determine the unadjusted and adjusted associations
34
35 140 between the AL and all-cause mortality. Age, sex, education, and marital status were included
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37 141 in the demographically adjusted models; smoking status, physical exercise, and chronic status
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39 142 including pulmonary disease and arthritis were added in the fully adjusted models. We modeled
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41 143 AL both continuously and in categories.
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47 145 Furthermore, one sensitivity analysis was undertaken, which aimed to exam if the model results
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49 146 were influenced when we did not use self-reported hypertension or diabetes to classify
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51 147 participants' risk category for BP or glucose.
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56 149 All tests were two-sided with a significance level of P-value less than 0.05. We conducted all
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58 150 analyses using STATA version 16.0 (Stata Corp, College Station, TX).
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152 **Study participants and public involvement**

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

157

158 **RESULTS**

159 **Distribution of Allostatic Load Categories**

160 The distribution of the AL score (range: 0-9) is right-skewed for both males and females; only
161 13 (1.8%) males and 10 (1.2%) females had a score of 6-9, respectively (Figure 1). The mean
162 AL score was 2.56 (SD=1.47) for males and 2.28 (SD=1.34) for females. For males, 25.5%,
163 64.2%, and 10.3% had an AL score of 0-1, 2-4, and 5-9, respectively. For females, 28.5%,
164 65.9%, and 5.6% had an AL score of 0-1 (low burden), 2-4 (medium burden), and 5-9 (high
165 burden), respectively.

166

167 **Demographic Characteristics**

168 A total of 709 (46.7%) males were included. The average age for males with an AL score of
169 0-1 (low burden), 2-4 (medium burden), 5-9 (high burden) was 77.6, 81.0, and 84.1 years,
170 respectively (P = 0.042). In addition, we observed significant differences in the prevalence
171 hypertension and diabetes by different AL burden among males.

172

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

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3 176 lower prevalence of smoking, and were less physically active. Females with a lower AL were
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5 177 more likely to be married and have any education than woman with higher AL score; they also
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8 178 had a lower prevalence of hypertension, diabetes, and heart disease. We did not observe
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10 179 significant difference in ethnicity, smoking, physical exercise, stroke, pulmonary disease,
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12 180 arthritis, and cancer across AL burden (low, medium, and high) among females.
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16 17 182 **Association between Allostatic Load and Mortality among Males**

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19 183 A total of 310 males died; the overall death rate was 105.7 per 1,000 person-years. Males with
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21 184 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
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23
24 185 201.3 per 1,000 person-years, respectively (Table 3).
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28 187 In the unadjusted Cox model, per unit higher AL score was significantly associated with a 75%
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30 188 higher hazard of death among males (95% CI: 44%, 112%; Table 3). The association slightly
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33 189 attenuated but persisted in the full adjusted model (hazard ratio [HR] = 1.51, 95% CI: 1.23,
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35 190 1.84). When modelled in categories, in the unadjusted model, the hazard of death of male with
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37 191 the medium AL burden (score: 2-4) was 1.68 times than hazard of death of those with a lower
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39 192 AL burden (score: 0-1); Male with a high AL burden (Score: 5-9) had a more than three-fold
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41 193 higher hazard of death than those with a lower AL burden (score: 0-1). These associations
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44 194 persisted after adjustment of socio-demographics and lifestyles. In the fully adjusted model,
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46 195 males with a medium AL burden (score: 2-4) had a 33% higher hazard of death than those with
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48 196 a low AL burden (score: 0-1). Males with a high AL burden (score: 5-9) had a more than two-
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50 197 fold higher hazard of death than those with a low AL burden (score: 0-1). Results did not
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53 198 change substantially in the sensitivity analyses (Tables S4).
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57 58 200 **Association between Allostatic Load and Mortality among Females**

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3 201 Over an average follow-up period of 3.9 years, 787 deaths (51.8%) occurred. A total of 477
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5 202 females died (58.9%); the overall death rate was 161.3 per 1,000 person-years. The death rates
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7 203 for females with an AL score of 0-1 (low burden), 2-4 (medium burden), and 5-9 (high burden)
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10 204 were 121.1, 174.8, and 252.6 per 1,000 person-years, respectively (Table 3).

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14 206 In the unadjusted Cox model, per unit higher AL (modelled continuously) was significantly
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16 207 associated with a 45% higher hazard of death among females (95% CI: 23%, 72%; Table 3).
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18 208 However, the association attenuated and became insignificant after adjusting for socio-
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20 209 demographics (age, sex, ethnicity, education, and marital status); similar results were observed
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22 210 when smoking, exercise, and chronic conditions including pulmonary disease and arthritis were
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24 211 additionally adjusted (HR = 1.16, 95% CI: 0.97, 1.38). When modelled in categories, the HR
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26 212 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
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28 213 2-4 and 5-9, respectively, compared with those with a score of 0-1 in the unadjusted model.
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30 214 After multivariable adjustment, females with an AL score of 2-4 and 5-9 had a 11% and 34%
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32 215 higher hazard of death, although the associations were not significant. Results did not change
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34 216 substantially in the sensitivity analyses (Tables S4).

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39 218 DISCUSSION

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42 219 The present study aimed to explore the association between AL burden and all-cause mortality
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44 220 among men and women aged at least 60 years in China. This finding is somewhat consistent
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46 221 with previous evidence suggesting that AL may be predictor of all-cause mortality later in life
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48 222 [7,19]. In addition, we found that older men with high AL burden had a more than two-fold
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50 223 hazard of death than those with a low AL burden. There is no significant association observing
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52 224 among females, but the findings are trending in the expected direction. These findings were in
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54 225 line with previous studies showing men tend to have higher AL with higher risk of death than
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3 226 women, and gender difference among AL score and cause-specific mortality risk including
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5 227 infectious diseases, cardiometabolic disease, and malignant neoplasm [7, 30, 31]. One possible
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7 228 explanation for the sex differences in the association between AL and mortality among older
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9 229 adults is that older women might be less vulnerable to stress men due to sex differences in the
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11 230 hippocampal formation in humans [32, 33, 34]. In addition, it has been shown that estrogen
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13 231 plays an important role in the brain with the development of aging, aiming to maintain allostasis
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15 232 when facing physiological stress, which may be possible to protect women against age-related
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17 233 diseases [35,36,37]. Moreover, Gruenewald et al. (2006) stated that gender difference in
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19 234 forecasting mortality risk among older people by biomarkers; compared with female,
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21 235 neuroendocrine and immune related biomarkers were more predictive in males [38]. In addition,
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23 236 the sex difference in the association between AL and mortality may be explained by behavioral
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25 237 factors. Social support is effective in relieving stress [39]. Women are more socially active to
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27 238 seek emotional support when facing stress than men [39]. Furthermore, in our study, women
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29 239 had high mean age, less education, lower prevalence of smoking, and less exercise. Therefore,
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31 240 adjustment of these covariates may influence the significant level of association in the final
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33 241 model.
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42 243 To our knowledge, our study was the first to reveal a significant association between AL and
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44 244 mortality among male participants only. However, we need to interpret these results with
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46 245 caution because the findings regarding the association between AL and mortality among
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48 246 females were trending in the expected direction. A study with more female participants is
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50 247 needed to provide a more definite conclusion. Previous study has identified increased risk of
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52 248 all-cause mortality associated with increasing AL score in men [7]. We found that the strength
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54 249 of the association differed between the present study and Hwang et al.'s work among men.
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56 250 There are several plausible explanations for this discrepancy. First, the population in Hwang et
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3 251 al.'s study (≥ 54 years) was younger than ours (≥ 60 years). Second, we did not include cortisol,
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5 252 which is a commonly used indicator of the primary mediator stress, due to data unavailability.
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7 253 Surrogate measures were used in the present study, which may lead to weaker associations.
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9 254 Third, two studies used different cut-points for constructing the AL score. We used sex-specific
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11 255 cut-off points for each biomarker, while general cut-points were used in Hwang et al.'s work.
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13 256 Fourth, follow-up length was different between the two studies. Furthermore, different choices
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15 257 of model covariates may influence the strength of observed associations. Hwang et al.'s
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17 258 research only adjusted for age and sex.
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24 260 For the sensitivity analysis of the association between AL and mortality, the overall magnitude
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26 261 of the HR was not largely altered, and the association was still statically significant. This
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28 262 suggests that the results of the association between AL category and mortality may not
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30 263 influenced by the participants who were self-reported disease status, which may support that
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32 264 our results are robust (Table S4).
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38 266 The AL was initially constructed using primary mediator stress including cortisol, epinephrine,
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40 267 and norepinephrine. These biomarkers are not widely available and secondary responses of
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42 268 cardiovascular, inflammatory and metabolic biomarkers such as CRP, BP and heart rate were
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44 269 used as surrogate measures. Although multiple CVD risk factors were included, AL, in theory,
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46 270 represents multisystem physiological dysregulation instead of functional decline in one system.
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48 271 Previous studies showed that AL was able to stratify the risk of a wider range of health
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50 272 outcomes than traditional CVD risk factors [4], it appears that AL could predict more health
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52 273 information including CVD incident, decline of cognition function, decline of physical
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54 274 function, and mortality than traditional CVD risk factors. The current study did not have data
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3 275 on primary mediator of AL, so we did not conduct separate analysis to investigate the
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5 276 association between primary vs. secondary mediators of AL and mortality.
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10 278 This study has some strengths. This is among the first to investigate the association between
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12 279 AL and mortality using a Chinese population. Moreover, our study used of CLHLS dataset that
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14 280 is a large nationally representative old population survey in China. Furthermore, we updated
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16 281 the quartile risk method for biomarkers BMI, total cholesterol, and triglyceride due to inversely
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18 282 association with mortality among older adults, which may more truly reflect AL score in old
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20 283 people. Additionally, our study added evidence to support sex differences in the association
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22 284 between AL and the increased risk of mortality. Finally, sex-specific cut-off points were used
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24 285 to construct the AL score may more truly reflect association between AL and mortality in
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26 286 different gender compared to previous study. Additionally, we collected detailed covariates
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28 287 information including age, sex, marital status, ethnicity, education, chronic diseases, which
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30 288 enable us to adjust for a range of potential confounders in the final cox model.
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38 290 Despite these strengths, we acknowledge some limitations. First, we did not include any
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40 291 primary neuroendocrine biomarkers such as cortisol in constructing the AL score due to data
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42 292 unavailability; this might partially explain the null finding regarding the association between
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44 293 AL and mortality among women. The cortisol biomarker plays an important role in responding
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46 294 stress, which needs repeated measurements within 1-2 days which causes difficulties to
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48 295 measure in large national survey [40]. Inclusion of cortisol biomarker maybe improve our
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50 296 power of AL score predictions for mortality. Additionally, we classified AL biomarkers based
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52 297 on sex-specific quartiles; however, these measures may vary over time, leading to
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54 298 misclassification. Furthermore, there is huge loss to follow up (>20%; 552 of 2439), although
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56 299 only 3 to 7-year range of follow up (2011 to 2014-18), which may underestimate the association.
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3 300 Then, participants who provided blood sample in this study were residents in eight longevity
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5 301 areas including Laizhou of Shandong Province, Xiayi of Henan Province, Zhongxiang of Hubei
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7 302 Province, Mayang of Hunan Province, Sanshui of Guangdong Province, Yongfu of Guangxi
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9 303 Autonomous Region, Chengmai of Hainan Province, Rudong of Jiangsu Province, which were
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11 304 from 8 of 23 provinces, five autonomous regions, four municipalities, and two special
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13 305 administrative regions. Therefore, our results may not be greatly generalizable to older adults
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15 306 living in other regions of China. Lastly, it is important to notice that the sample was extremely
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17 307 old, which may influence the magnitude of the association presented.
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24 309 Therefore, even though we found that higher AL burden was associated with increased all-
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26 310 cause mortality among Chinese men aged at least 60 years, but not women, it is really
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28 311 recommended that these results need to be replicated in large longitudinal studies with longer
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30 312 follow-up time, with more AL biomarkers such as cortisol, or with containing Chinese from
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32 313 more regions apart from eight longevity areas. This would helpful for comparing with our
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34 314 results and validating them in different populations.
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39 40 316 CONCLUSION

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42 317 In conclusion, our study showed that higher AL burden was associated with increased all-cause
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44 318 mortality among Chinese men aged at least 60 years. We did not find strong evidence among
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46 319 women. Intervention programs targeting modifiable components of the AL burden may help
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48 320 prolong lifespan for older adults, especially men, in China.
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3 323 **Abbreviation:**
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5 324 AL: allostatic load; DHEA-S: dehydroepiandrosterone sulphate; BP: blood pressure; CLHLS:
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7 325 Chinese Longitudinal Healthy Longevity Survey; SBP: systolic BP; DBP: diastolic BP; BMI:
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9 326 body mass index; HDL: total cholesterol, high density lipoprotein; CRP: C-reactive protein.
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14 328 **DECLARATIONS:**
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17 329 **Ethics approval and consent to participate:**
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19 330 The original CHARLS was approved by the Ethical Review Committee of Peking University.
20

21 331 The baseline data collection was obtained from the Biomedical Ethics Review Committee of
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23 332 Peking University (IRB00001052-11015), and all participants have signed informed consent.
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28 334 **Consent to publish:**
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30 335 Not required
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35 337 **Availability of data and materials:**
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37 338 The raw data are available on website:
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39 339 <https://opendata.pku.edu.cn/dataset.xhtml?persistentId=doi:10.18170/DVN/WBO7LK>
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46 342 Authors have no conflict of interests to disclose.
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49 343
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11
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13
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15
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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

^aMarried vs. widowed, never married, and divorce.

Table 3.

Association between allostatic load and mortality among males and females.

Males (N = 709)				
		Unadjusted	Demographically adjusted ^a	Fully adjusted ^b
Events per 1,000 PYs (95% CI)		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)				
		Unadjusted	Demographically adjusted ^a	Fully adjusted ^b
Events per 1,000 PYs (95% CI)		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.

^aDemographically adjusted model included age, ethnicity (Han vs. minority), education (any vs. none), and marital status (married vs. others).^bFully 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.

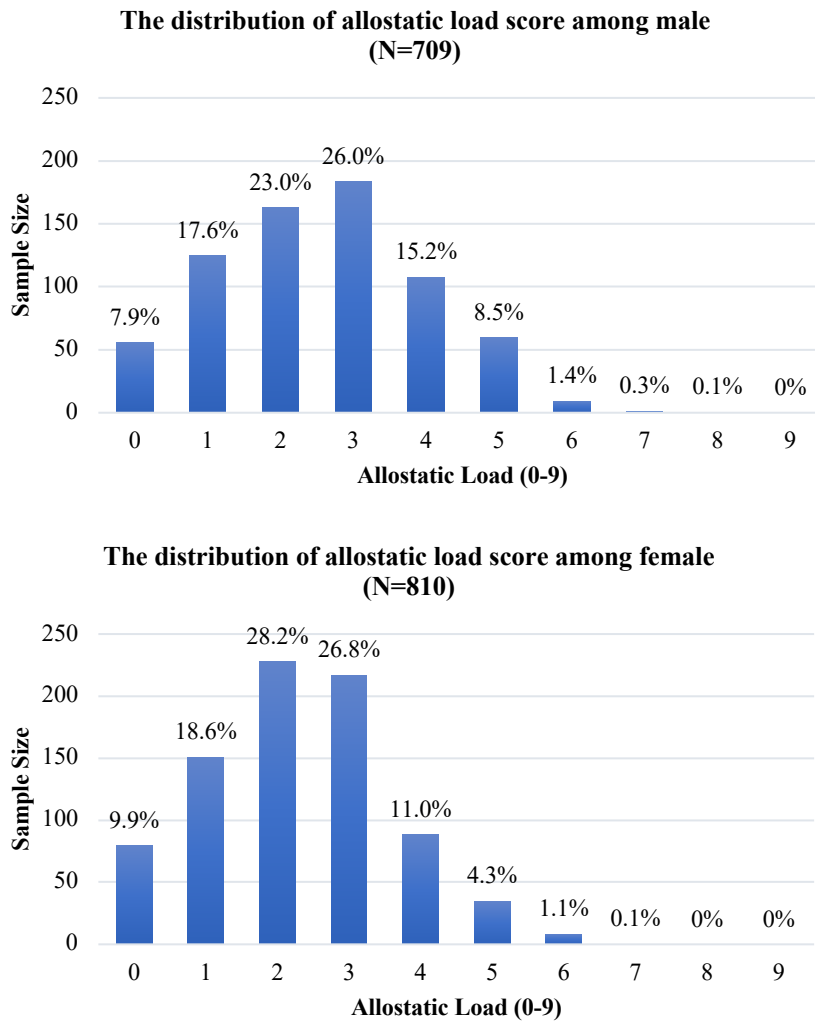
Figure 1. Distribution of sample size by allostatic load score among males and females.

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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

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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. (%)			
Current Smoker	295 (19.5%)	170 (18.7%)	0.460
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

^aMarried vs. widowed, never married, and divorced.

^bExcluded 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

^aMarried 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.

^aDemographically adjusted model included age, ethnicity (Han vs. minority), education (any vs. none), and marital status (married vs. others).

^bFully 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.

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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

1	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	11-12
2			(b) Report category boundaries when continuous variables were categorized	
3			(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
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9	Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	11-12
10				
11	Discussion			
12				
13	Key results	18	Summarise key results with reference to study objectives	12
14	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
15				
16	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
17				
18				
19	Generalisability	21	Discuss the generalisability (external validity) of the study results	15
20				
21	Other information			
22	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
23				
24				
25				

*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>.