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Allostatic load as predictor of mortality: a cohort study from Lolland-Falster, Denmark

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ABSTRACT

Objectives The purposes of the present study were to determine the association between (1) 10 individual biomarkers and all-cause mortality; and between (2) allostatic load (AL), across three physiological systems (cardiovascular, inflammatory, metabolic) and all-cause mortality.

Design Prospective cohort study.

Setting We used data from the Lolland-Falster Health Study undertaken in Denmark in 2016–2020 and used data on systolic blood pressure (SBP) and diastolic blood pressure (DBP), pulse rate (PR), waist–hip ratio (WHR) and levels of low-density lipoprotein cholesterol (LDL-c), high-density lipoprotein cholesterol (HDL-c), triglycerides, glycated haemoglobin A1c (HbA1c), C-reactive protein (CRP) and serum albumin. All biomarkers were divided into quartiles with high-risk values defined as those in the highest (PR, WHR, triglycerides, HbA1c, CRP) or lowest (HDL-c, albumin) quartile, or a combination hereof (LDL-c, SBP, DBP). The 10 biomarkers were combined into a summary measure of AL index. Participants were followed-up for death for an average of 2.6 years.

Participants We examined a total of 13 725 individuals aged 18+ years.

Primary outcome measure Cox proportional hazard regression (HR) analysis were performed to examine the association between AL index and mortality in men and women.

Results All-cause mortality increased with increasing AL index. With low AL index as reference, the HR was 1.33 (95% CI: 0.89 to 1.98) for mid AL, and HR 2.37 (95% CI: 1.58 to 3.54) for high AL.

Conclusions Elevated physiological burden measured by mid and high AL index was associated with a steeper increase of mortality than individual biomarkers.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ Analysis based on a large population-based health study.
⇒ Complete follow-up for death via linkage with Danish Civil Registration System.
⇒ Biomarkers from only one point in time.
⇒ No biomarker from neuroendocrine system available.

INTRODUCTION

Biological markers (biomarkers) were originally defined as 'cellular, biochemical or molecular alterations that are measurable in biological media such as cells, human tissues or fluids'.[1] Later the definition was extended to include 'indicators of normal biological processes, pathogenic processes and pharmacological responses to therapeutic interventions'.[2] In clinical settings, measurement of biomarkers in blood samples is used to detect and diagnose medical conditions. Biomarkers as independent predictors of all-cause mortality are therefore of considerable clinical and research interest;[3] dyslipidaemia including high levels of triglycerides and low-density lipoprotein cholesterol (LDL-c), and low levels of high-density lipoprotein cholesterol (HDL-c), have been reported to be independent risk factors for all-cause mortality.[4–6] Lower levels of albumin[7] and higher levels of C-reactive protein (CRP),[8] and glycated haemoglobin A1c (HbA1c)[9] have likewise been linked to mortality. Also, there is some evidence that the relationship between some of these biomarkers and all-cause mortality varies across sex and age groups.[10 11]

The concept of allostatic load (AL) refers to the 'wear and tear' of the body resulting from repeated stimulation of stress responses via the hypothalamic–pituitary–adrenal axis and the sympathetic-adrenal-medullary system.[12] As a latent variable, AL cannot be directly measured but it can be estimated using an AL index, which is composite of biomarkers from multiple organ systems integrated into a single score. The first AL developed by Seeman *et al* in 1997 included 10 biomarkers monitoring various physiological systems.[13] However, the type and number of biomarkers used in published studies have ranged from 6 to 24.[14] The most frequently used Al construct, originally proposed by Gruenewald *et al* in 2012,[15]

includes 24 biomarkers. It has been suggested that in the calculation of AL, the threshold of risk for each biomarker should be obtained by the quartiles or quintiles of the values of the biomarker.[16] AL has been reported to be a better predictor of mortality than individual biomarkers, however, there are still gaps in the understanding of the associations.[17 18] AL has been suggested also as a tool for allocation of nursing resources.[19]

This study provides data from the Lolland-Falster Health Study (LOFUS),[20] a population-based survey undertaken in 2016–2020 in Lolland-Falster, a rural–provincial region in Denmark with a life expectancy much below the national average,[21] and with health problems reported more frequently than in the rest of the country.[22] Using the LOFUS data, the purposes of the present study were (1) to determine the association between 10 individual biomarkers and all-cause mortality; and (2) to examine the association between AL, across three physiological systems (cardiovascular, inflammatory, metabolic system) and all-cause mortality. The hypothesis is that AL can be used as an informative tool in predicting future risk of death in the general adult population.

METHODS

Study population

We undertook a prospective cohort study of participants from LOFUS; a household-based population study with data collected between February 2016 and February 2020. Persons aged 18 years and above were randomly sampled from the Danish Civil Registration System and invited to participate together with the rest of their households. Participation required informed consent. A detailed description of the study protocol[20] and information on the socioeconomic determinants of participation[23] have been published previously. Persons below 18 years, and pregnant women were excluded from the present study.

Patient and public involvement

Patients were not actively involved in any stage of the present study. Once the paper has been published in the international literature, the key results will be reported also in the local press.

Self-reported data

From questionnaires, we used data on smoking (never, former, current), and presence of chronic conditions (cardiovascular disease, diabetes, cancer) at the time of participation in LOFUS.

Biomarkers

Non-fasting blood samples were collected in vacutainer blood collection tubes (Becton, Dickinson and Company; Franklin Lakes, New Jersey, USA) and kept at room temperature until same day analysis at the Department of Clinical Biochemistry at Nykøbing Falster Hospital, accredited by the International Organization for Standardization 15189. We used data on HDL-c, LDL-c, triglycerides, albumin, CRP and HbA1c. LDL-c was calculated by using Friedewald formula[24] when the plasma triglyceride concentration was below 4.5 mmol/L. Systolic (SBP) and diastolic (DBP) blood pressure were based on three consecutive digital measurements on the upper left arm (apparatus type Welch Allyn Connex proBPO 3400). The mean values of the second and third measurement were used in this study (only one measurement was used if the other was missing). Waist-hip ratio (WHR) was calculated by waist-circumference divided by hip circumference. Body mass index (BMI) was defined as weight in kilograms divided by height in metres squared (kg/m^2).

In the calculation of AL, biomarkers are most often dichotomised into low and high values based on either a percentile or a predetermined cut-off value.[16] However, before doing so, we mapped for each biomarker the association between level of the marker and all-cause mortality, see Method below. For most biomarkers the association was monotonic (see online supplemental figure 1). These biomarkers were then dichotomised according to the sex-specific and age-specific quartiles. For age, we dichotomised at age 60. Some previous studies focused on AL in people aged 60 and above[25 26] and we intuitively found it reasonable to distinguish in the same way between 'young' and 'old' people in our data; age 60 was furthermore the median age of our study population; and with this age-dichotomisation we avoided violations of the model assumption in the statistical analysis. We dichotomised biomarkers with high-risk values defined as those in the highest quartile of the sex-specific and age-specific distribution, except for HDL-c and albumin, where the lowest quartile was the high-risk value. For LDL-c, SBP and DBP the associations were U-shaped, and the high-risk values for these biomarkers were therefore defined as including both the lower and the upper quartiles (see online supplemental table 1). For biomarkers with U-shaped associations, we tested out also using octiles as cut-off points. However, this resulted in some violations of the model assumptions in the statistical analysis, and for SBP the upper octile cut-off was from a clinical point of view very high. On this basis we used the quartile cut-offs also for the biomarkers with the U-shaped association. For all biomarkers, the highest and lowest quartile of risk scores were either lower or similar to clinical cut-points.[27–31]

BMI was divided into underweight (BMI less than 18.5) normal (BMI 18.5–24.9), overweight (BMI 25.0–29.9) or obese (BMI 30.0 or greater); reported diseases into either present or not; and smoking status into never, former or current.

Allostatic load scores

The AL scores were computed using biomarkers from: the cardiovascular system (CVS) (SBP, DBP and pulse rate (PR)); the metabolic system (MS) (LDL-c, triglycerides, HDL-c, WHR, HbA1c); and the inflammatory system (IS) (CRP, serum albumin).

Each system-specific AL score was then defined as the number of biomarkers with a high-risk value, hence as an integer value between 0 and 3 for the CVS, 0 and 5 for the MS and 0 and 2 for IS. The AL index was defined as the sum of all scores and divided in three groups based on tertiles contrasting individuals with (AL: 0–2), mid (AL: 3–4) and high (AL: 5–10). Note that, all biomarkers were given equal weight in accordance with previous studies.[16 18]

All-cause mortality

LOFUS participants were followed-up for death with data obtained from the Danish Civil Registration System on 26 February 2021.

Data management and statistical analyses

Observations with missing values in any of the variables were excluded from the analyses (1989 out of 15 714, ie, 12.6%, see online supplemental table 2). Values below the lower limit of detection were replaced with random numbers sampled with replacement from the set $\{k \times 10 \wedge (-n), k = 1, \cdots, L\}$, where n is the variable-specific number of decimals reported in the data and $L \times 10 \wedge (-n)$ the limit of detection (see online supplemental table 3).

Participants were followed-up from date of participation in the LOFUS study until date of death or end of follow-up on 26 February 2021, whichever came first. In order to define the biomarkers' high-risk values, we first studied the association between levels of each individual biomarker and mortality, allowing for possible non-linear relations. This analysis was carried out via Cox proportional-hazard models with biomarker levels as continuous covariates, modelled with natural cubic splines with 2 df (except for LDL-c, where 3 df were used), and further adjusting for sex and age. By graphical inspection, a U-shaped association was found for LDL-c, SBP and DBP (see online supplemental figure 1). Therefore, for these biomarkers both the sex and age-specific (ie, below or above age 60) lower and upper quartiles were defined as high risk, while only one quartile for the others (upper or lower, in accordance with the existing literature) (see online supplemental table 1).

Associations between all-cause mortality and dichotomised biomarkers levels (low/high risk), system-specific AL scores and total AL index, were modelled with Cox proportional-hazard models. Here, we present two models: Model 1, where HRs are adjusted for sex and age; Model 2, where results are further adjusted for BMI, prevalent diseases and smoking status. HRs for the individual biomarkers (table 1) and for system-specific AL scores (table 2) are mutually adjusted. Proportional hazards assumptions in the above models have been tested using Schoenfeld residuals. Numbers below 5 are not reported. In addition, we report HRs for a one-point increase in the AL index.

Data management, statistical analyses and plots were done in R V.4.0.3,[32] with packages splines,[32] survival,[33] tidyverse,[34] ggrepel[35] and ggpubr.[36]

RESULTS

The LOFUS database used for this study included 13 725 persons, of whom 53% were women and 47% men. The median follow-up time was 2.6 years (IQR 1.5) and the median age was 57.6 in women and 59.9 in men. One-fourth of the participants were obese, and one-fifth were current smokers. Presence of cardiovascular disease at the time of LOFUS participation was reported by 28%, diabetes by 5% and cancer by 4%. On the value of total AL index, participants were divided approximately into tertiles; 32% low, 40% mid and 38% high. During the

Table 1 Multivariate Cox proportional hazard regression of all-cause mortality for Lolland-Falster Health Study participants by individual biomarkers

Variable	Non exposed	Exposed	HR (95% CI) Model 1*	HR (95% CI) Model 2†
HDL cholesterol, mmol/L	High	Low	1.22 (0.88 to 1.69)	1.24 (0.89 to 1.73)
LDL cholesterol, mmol/L	Mid	High and low	1.22 (0.91 to 1.62)	1.13 (0.85 to 1.51)
Triglycerides, mmol/L	Low	High	0.93 (0.66 to 1.32)	0.94 (0.67 to 1.33)
Albumin, g/L	High	Low	1.55 (1.17 to 2.07)	1.54 (1.16 to 2.06)
CRP, mg/L	Low	High	1.42 (1.05 to 1.92)	1.41 (1.04 to 1.91)
HbA1c, mmol/mol	Low	High	1.25 (0.93 to 1.68)	1.24 (0.90 to 1.71)
Systolic blood pressure, mm Hg	Mid	High and low	1.20 (0.90 to 1.61)	1.17 (0.88 to 1.57)
Diastolic blood pressure, mm Hg	Mid	High and low	1.31 (0.98 to 1.76)	1.28 (0.95 to 1.72)
Pulse rate, PM	High	Low	1.34 (0.99 to 1.81)	1.23 (0.91 to 1.66)
Waist–hip ratio	Low	High	1.02 (0.74 to 1.41)	1.08 (0.76 to 1.52)

*Adjusted for age and sex.
†Additionally adjusted for body mass index, reported diseases and smoking status.
CRP, C-reactive protein; HbA1c, glycated haemoglobin A1c; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PM, per minute.

Table 2 Multivariate Cox proportional hazard regression of all-cause mortality for Lolland-Falster Health Study participants by allostatic load index

Variable	Reference	Level	HR (95% CI) Model 1*	HR (95% CI) Model 2†
Allostatic load index	Low	Mid	1.39 (0.94 to 2.06)	1.33 (0.89 to 1.98)
		High	2.45 (1.68 to 3.59)	2.37 (1.58 to 3.54)
Continuous allostatic load measure			1.23 (1.14 to 1.32)	1.22 (1.13 to 1.32)
Inflammatory system score	Low	Mid	1.03 (0.74 to 1.44)	1.02 (0.73 to 1.42)
		High	2.39 (1.69 to 3.38)	2.38 (1.67 to 3.39)
Metabolic system score	Low	Mid	1.19 (0.76 to 1.86)	1.18 (0.75 to 1.85)
		High	1.54 (1.02 to 2.33)	1.54 (1.00 to 2.38)
Cardiovascular system score	Low	Mid	1.73 (1.08 to 1.78)	1.65 (1.02 to 2.65)
		High	2.06 (1.31 to 3.24)	1.89 (1.20 to 2.99)

*Adjusted for age and sex.
†Additionally adjusted for body mass index, reported diseases and smoking status.

follow-up period, 198 participants died; of these 39% were women and 61% men (table 3).

The multivariate Cox proportional hazard regression for individual biomarker and all-cause mortality, adjusted for sex and age and additionally for BMI, reported diseases and smoking, are listed in table 1. For all biomarkers, apart from triglycerides, a high-risk value was associated with an increased mortality level. However, only the HRs for low albumin and high CRP were statistically significantly elevated; HR 1.54 (95% CI: 1.16 to 2.06) and 1.41 (95% CI: 1.04 to 1.91), respectively.

The HR for all-cause mortality increased with increasing level of the AL from low as the reference over mid to high, table 2 and figure 1. For the IS AL score, the HR was 1.02 (95% CI: 0.73 to 1.42) for mid AL, and 2.38 (95% CI: 1.67 to 3.39) for high AL. For the MS AL score, the HRs were 1.18 (95% CI: 0.75 to 1.85) and 1.54 (95% CI: 1.00 to 2.38), respectively. For the CVS AL score, the HRs were 1.65 (95% CI: 1.02 to 2.65) and 1.89 (95% CI: 1.20 to 2.99), respectively. The gradient for the total AL index was a HR of 1.33 (95% CI: 0.89 to 1.98) for mid AL, and 2.37 (95% CI: 1.58 to 3.54) for high AL. HRs for one unit increase in AL (continuous AL) was 1.23 (1.14–1.32) when adjusted for age and sex, and 1.22 (1.13–1.32), when additionally adjusted for BMI, reported diseases and smoking status.

DISCUSSION

In this population-based study from a rural–provincial area of Denmark, we followed the adult population up for a median period of 2.6 years. High levels of individual biomarkers were overall associated with increased mortality, but most of them at a modest level of 20%–30%, and statistically significantly elevated for only CRP and albumin. High levels of physiological system-specific AL scores were associated with increased mortality at the level of 50%–140%; statistically significantly for the IS and CVS, and at borderline of significance for the MS. The composite measure of total AL index was a strong predictor of all-cause mortality. Persons with a high versus low total AL index had about 2.5 times the mortality. The total AL index was thus a better predictor of all-cause mortality than individual biomarkers and the MS and CVS AL scores, a pattern consistent with previous studies.[16 18 37]

The most comprehensive studies on AL and mortality all used data from the National Health and Nutrition Examination Survey (NHANES). Borrell *et al*[38] examined 12-year mortality by using data from 13 715 adults aged 25+ years of whom 2491 had died. They calculated AL based on nine biomarkers; albumin, CRP, total cholesterol, HDL-c, HbA1c, WHR, SBP, DBP and PR. Using a clinical cut-off AL score, they found that, compared with persons with an AL score of ≤1, those with AL scores of 2 and 3+ had adjusted HRs of 1.40 (95% CI: 1.11 to 1.76) and 1.88 (95% CI: 1.56 to 2.26), respectively.

Levine and Crimmins[39] examined 10-year all-cause and disease-specific mortality. In total, 15 042 persons were eligible, but biomarker data were available for only 9942 adults aged 30+, of whom 1076 had died. They included data on albumin, CRP, WHR, total cholesterol, HDL-c, HbA1c, PR, SBP and DBP. For each of the nine biomarkers, a person was classified as high or low based on clinical cut-off points, and the AL score was the number of biomarkers classified as high. In addition, an expanded AL score included 5 additional biomarkers defined by quintiles; and a continuous AL score used a continuous z-score measure for all 14 biomarkers. For the first AL score, a HR of 2.75 (p<0.001) was found for all-cause mortality when persons with the highest quintile of AL were compared with those with the lowest. Somewhat stronger gradients were found for the expanded; 3.62 (p<0.0001) and continuous; 6.97 (p<0.0001), ALs.

Howard and Sparks[40] studied 11 733 participants from NHANES. Imputation was used to estimate missing values. Their AL measure was based on DBP, SBP, PR, total cholesterol, HDL-c, triglycerides, HbA1c, BMI,

Table 3 Lolland-Falster Health Study (LOFUS). Baseline characteristics of study population and deaths in follow-up period, n(%). For definition of cut-off values, see online supplemental table 1

Characteristics	Females	Males	Total	Female death	Male death	Total death
Population	7270 (53)	6455 (47)	13 725 (100)	78 (39)	120 (61)	198 (100)
Follow-up time, median (IQR)	2.6 (1.6)	2.7 (1.5)	2.6 (1.5)	2.0 (1.6)	1.9 (1.8)	1.9 (1.8)
Median age (IQR)	57.6 (21.9)	59.9 (21.6)	58.7 (22.0)	70.5 (16.4)	74.0 (15.2)	72.8 (16.2)
BMI, kg/m^2						
Underweight	134 (1.8)	42 (0.7)	176 (1.3)	5 (6.4)	Not reported	6 (3.0)
Normal weight	3038 (41.8)	1862 (28.8)	4900 (35.7)	29 (37.2)	40 (33.3)	69 (34.8)
Overweight	2335 (32.1)	2940 (45.5)	5275 (38.4)	26 (33.3)	52 (43.3)	78 (39.4)
Obese	1763 (24.3)	1611 (25.0)	3374 (24.6)	18 (23.1)	27 (22.5)	45 (22.7)
Smoking						
Never	3586 (49.3)	2737 (42.4)	6323 (46.1)	21 (26.9)	24 (20.0)	45 (22.7)
Former	2342 (32.2)	2425 (37.6)	4767 (34.7)	32 (41.0)	70 (58.3)	102 (51.5)
Current	1342 (18.5)	1293 (20.0)	2635 (19.2)	25 (32.1)	26 (21.7)	51 (25.8)
Chronic conditions						
Cardiovascular disease reported	1828 (25.1)	1999 (31.0)	3827 (27.9)	42 (53.8)	60 (50.0)	102 (51.5)
Diabetes reported	264 (3.6)	440 (6.8)	704 (5.1)	9 (11.5)	15 (12.5)	24 (12.1)
Cancer reported	245 (3.4)	275 (4.3)	520 (3.8)	13 (16.7)	24 (20.0)	37 (18.7)
Cardiovascular system						
Systolic blood pressure						
Low risk	3548 (48.8)	3165 (49.0)	6713 (48.9)	32 (41.0)	52 (43.3)	84 (42.4)
High risk	3722 (51.2)	3290 (51.0)	7012 (51.1)	46 (59.0)	68 (56.7)	114 (57.6)
Diastolic blood pressure						
Low risk	3426 (47.1)	3164 (49.0)	6590 (48.0)	26 (33.3)	52 (43.3)	78 (39.4)
High risk	3844 (52.9)	3291 (51.0)	7135 (52.0)	52 (66.7)	68 (56.7)	120 (60.6)
Pulse rate						
Low risk	5366 (73.8)	4721 (73.1)	10 087 (73.5)	50 (64.1)	81 (67.5)	131 (66.2)
High risk	1904 (26.2)	1734 (26.9)	3638 (26.5)	28 (35.9)	39 (32.5)	67 (33.8)
AL cardiovascular system score						
Low	1815 (25.0)	1506 (23.3)	3321 (24.2)	7 (9.0)	16 (13.3)	23 (11.6)
Mid	2117 (29.1)	2154 (33.4)	4271 (31.1)	27 (34.6)	43 (35.8)	70 (35.4)
High	3338 (45.9)	2795 (43.3)	6133 (44.7)	44 (56.4)	61 (50.8)	105 (53.0)
Metabolic system						
HDL-c						
Low risk	4934 (67.9)	4706 (72.9)	9640 (70.2)	46 (59.0)	85 (70.8)	131 (66.2)
High risk	2336 (32.1)	1749 (27.1)	4085 (29.8)	32 (41.0)	35 (29.2)	67 (33.8)
Triglycerides						
Low risk	5299 (72.9)	4761 (73.8)	10 060 (73.3)	50 (64.1)	95 (79.2)	145 (73.2)
High risk	1971 (27.1)	1694 (26.2)	3665 (26.7)	28 (35.9)	25 (20.8)	53 (26.8)
HbA1c						
Low risk	5156 (70.9)	4438 (68.8)	9594 (69.9)	46 (59.0)	69 (57.5)	115 (58.1)
High risk	2114 (29.1)	2017 (31.2)	4131 (30.1)	32 (41.0)	51 (42.5)	83 (41.9)
Waist–hip ratio						
Low risk	5452 (75.0)	4831 (74.8)	10 283 (74.9)	57 (73.1)	85 (70.8)	142 (71.7)
High risk	1818 (25.0)	1624 (25.2)	3442 (25.1)	21 (26.9)	35 (29.2)	56 (28.3)
LDL-c						
Low risk	3459 (47.6)	2989 (46.3)	6448 (47.0)	31 (39.7)	51 (42.5)	82 (41.4)

Continued

Table 3 Continued

Characteristics	Females	Males	Total	Female death	Male death	Total death
High risk	3811 (52.4)	3466 (53.7)	7277 (53.0)	47 (60.3)	69 (57.5)	116 (58.6)
AL metabolic system score						
Low	1401 (19.3)	1249 (19.3)	2650 (19.3)	11 (14.1)	18 (15.0)	29 (14.6)
Mid	2413 (33.2)	2135 (33.1)	4548 (33.1)	18 (23.1)	37 (30.8)	55 (27.8)
High	3456 (47.5)	3071 (47.6)	6527 (47.6)	49 (62.8)	65 (54.2)	114 (57.6)
Inflammation system						
CRP						
Low risk	5451 (75.0)	4837 (74.9)	10288 (75.0)	51 (65.4)	73 (60.8)	124 (62.6)
High risk	1819 (25.0)	1618 (25.1)	3437 (25.0)	27 (34.6)	47 (39.2)	74 (37.4)
Albumin						
Low risk	4953 (68.1)	4655 (72.1)	9608 (70.0)	49 (62.8)	54 (45.0)	103 (52.0)
High risk	2317 (31.9)	1800 (27.9)	4117 (30.0)	29 (37.2)	66 (55.0)	95 (48.0)
AL inflammation system score						
Low	4027 (55.4)	3692 (57.2)	7719 (56.2)	41 (52.6)	42 (35.0)	83 (41.9)
Mid	2350 (32.3)	2108 (32.7)	4458 (32.5)	18 (23.1)	43 (35.8)	61 (30.8)
High	893 (12.3)	655 (10.1)	1548 (11.3)	19 (24.4)	35 (29.2)	54 (27.3)
Total AL index						
Low	2306 (31.7)	2112 (32.7)	4418 (32.2)	14 (17.9)	24 (20.0)	38 (19.2)
Mid	2882 (39.6)	2599 (40.3)	5481 (39.9)	26 (33.3)	45 (37.5)	71 (35.9)
High	2082 (28.6)	1744 (27.0)	3826 (27.9)	38 (48.7)	51 (42.5)	89 (44.9)

AL, allostatic load; BMI, body mass index; CRP, C-reactive protein; HbA1c, glycated haemoglobin A1c; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol.

albumin and CRP. They found that a one-unit increase in AL represented a 7% increase in risk of death when adjusted for age, sex, ethnicity, socioeconomic status and health behaviour.

The National Child Development Study was followed-up for deaths from birth in 1958 to 1 December 2013, that is, to the age of 55 years.[18] AL based on 10 biomarkers was calculated and divided into three levels. All-cause mortality for persons with mid or high AL was compared with that of persons with low AL, and adjusted for early life, childhood, young and adulthood confounders. The HR of death was 1.71 (95% CI: 1.07 to 2.72) for persons with mid AL, and 2.57 (95% CI: 1.59 to 4.15) for those with high AL. The association between AL and all-cause mortality was stronger than the associations between of the individual 10 biomarkers and all-cause mortality.

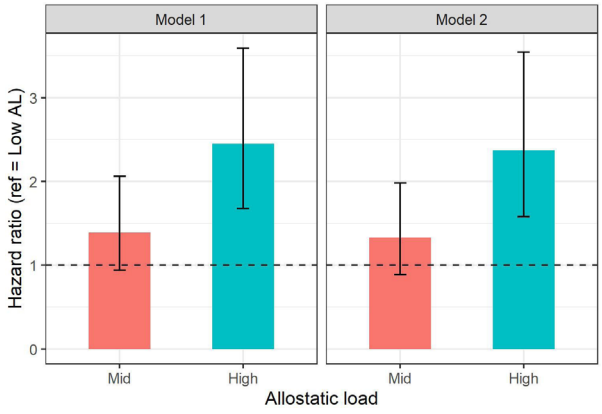

Figure 1 All-cause mortality by level of allostatic load index, as HR (95% CI). AL, allostatic load.

The NHANES studies vary in number of participants included in the studies, in length of follow-up for mortality, in biomarkers included, in the definition of AL and in methods used for AL calculation. Nevertheless, all the studies indicated that all-cause mortality increased with increasing AL. The study by Borell *et al*[38] is the one methodologically most similar to our study and the gradient of 1.88 (95% CI: 1.56 to 2.26) is compatible with the 1 of 2.37 (95% CI: 1.58 to 3.54) found in our study, and so is the gradient of 2.57 (95% CI: 1.59 to 4.15) found in the National Child Development Study.

For individual biomarkers in our study, HRs were highest for CRP and albumin. CRP is the prototypical acute-phase response protein that increases during systemic inflammation,[41] while albumin is a major component of plasma protein, required for transportation and to maintain oncotic pressure, acid–base function, microvascular permeability and to prevent platelet aggregation.[42] Inflammation increases capillary permeability and thereby escape of serum albumin, leading to expansion of interstitial space and increasing the distribution volume of albumin causing lower serum albumin concentrations.

High level of CRP and low level of albumin have thus previously been linked with a variety of health outcomes including morbidity and mortality.[7 8 43]

We found a U-shaped association between LDL-c and mortality. Elevated LDL-c is a well-established risk factor of atherosclerosis and cardiovascular disease, and the general perception is that high level of LDL-c is associated with an increased risk of morbidity and mortality.[44 45] Nevertheless, studies on the association between LDL-c levels and mortality have provided conflicting results. Some studies found increasing level of LDL-c to be associated with lower mortality,[546] and some studies found no association.[45 47 48] However, most studies were conducted in elderly people often with an intake of lipid-lowering agents. A more recent study in young Koreans found an association between low level of LDL-c and an increased risk of cancer, cardiovascular and all-cause mortality.[49] These findings were supported by a Chinese study of participants aged 40+ years.[50] A recent Danish study among 108 243 individuals aged 20–100 years found the lowest all-cause mortality at an LDL-c concentration of 3.6 mmol/L (140 mg/dL), and higher mortality at both lower and higher levels.[51] Our findings for LDL-c were thus in accordance with these recent observations. Seplaki *et al* suggested that both high and low ends of the risk continuum for the construct of AL could be more informative than simply using high-risk quartiles. They assigned a value of '1' for values above the 75th percentile and below the 25th percentile of the distribution, and a value of '0' for intermediate values.[52]

We found both higher and lower levels of DBP to be associated with an increased mortality, and a similar tendency was indicated for SBP. The association between lower BP and mortality is still of discussion.[53–55] Most studies have found this association among elderly people and linked it to chronic disease, for example, cardiovascular disease (cardiac failure or ischaemic heart disease), cancer, poor functional status or frailty. Low BP has also been associated with poor function and low quality of life,[56 57] but in previous studies only the highest quartile or the clinical cut-off value have been used as predictor of all-cause mortality.

Several methods have been used to define an AL composite index, including the count-based, canonical correlation, z-score and grade of membership method.[58 59] The most commonly used method is the count-based method, where a summary index is calculated by summing the number of biomarkers falling within the high-risk category, either defined by the percentile (ie, upper or lower 25th percentile of the sample's distribution) or by the clinical cut-off value. In our analysis with the two-tail cut-off points, we found HRs for LDL-c of 1.13 (95% CI: 0.85 to 1.51); for SBP of 1.17 (95% CI: 0.88 to 1.57; and for DBP of 1.28 (95% CI: 0.95 to 1.72). If we have used instead the single high-risk quartile cut-off point, we would have found HRs for LDL-c of 0.71 (95% CI: 0.49 to 1.03); for SBP of 0.96 (95% CI: 0.68 to 1.35) vs), and for DBP of 1.24 (95% CI: 0.86 to 1.81). The two-tail cut-off points thus provided a better identification of persons with high mortality than the one-tail cut-off points.

The issue of whether a clinical or sample-based cut-off criteria should be used is still of discussion,[17] however, studies comparing distinct measurement approaches have found only modest differences in their predictive utility.[15 60 61]

Strengths and limitations

The strengths of our study included the size of the cohort in terms of the large number of individuals recruited from a general adult population, and the complete follow-up for death by linkage with the Danish Civil Registration System.

Our study also had some limitations. First, the choice of biomarkers used to construct the AL index. The AL theory emphasises the importance of measuring dysregulation across different physiological systems, including biomarkers from the neuroendocrine, CVS, MS and IS.[13] The neuroendocrine system (stress response) is believed to play a key role in allostasis and subsequent AL, as a series of physiological changes takes place before initial stress responses occur (such as rapid increases in blood sugar and BP that supply the body with additional energy). However, biomarkers from the neuroendocrine system are difficult to measure, as repeated measurements over 1–2 days are recommended. These requirements cannot be fulfilled in population studies, where participants are examined only once, and biomarkers from the neuroendocrine system were therefore not available for our study.

Second, the initial stress responses are followed by secondary outcomes from the MS, IS and CVS, and these markers were all available in our data. Nevertheless, greater sensitivity could have been achieved by studying the dynamic changes over time in these markers to fully capture the flexibility of stress response mechanisms across the lifespan.

Finally, differences across studies in construction of AL indices could influence the comparison of results. We used the shape of the association between level of a given biomarker and all-cause mortality as the basis for the categorisation of the biomarker into low and high values. One can argue therefore that our analysis was circular in the way that we used outcome on the dependent variable to categorise levels of the independent variable. We believe that this was justifiable in the context here where the purpose was to optimise the predictive power of the AL index. However, validation in other data sets are needed before our approach can be recommended for research in general and for eventual clinical use.

Conclusion

Our findings demonstrated that an optimally constructed AL index was a strong predictor of all-cause mortality. This supported the conceptual validity of AL as an effective marker of the cumulative physiological burden on the body. These findings can contribute to the evidence for the use of an AL index as a basis for targeted efforts

to bring down continued stress exposures, and in this way prevent the potential detrimental effect of these exposures on health. Our findings on the U-shaped association with LDL-c, DBP and SBP and all-cause mortality suggested that AL measures incorporating risks at both the low and the high end of biomarkers may yield the best prediction of all-cause mortality.

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Contributors All authors contributed significantly to the study. RJ provided the LOFUS data. NEB-R, EL and GN designed the study, interpreted the data and drafted the manuscript. GN performed the statistical analysis. CE, CC, RJ, KR and SEB contributed to the interpretation and writing of the manuscript. All authors critically revised and approved the final manuscript. EL is the guarantor.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

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