Socio-Environmental, Lifestyle, Behavioural, and Psychological Determinants of Biological Ageing: The Singapore Longitudinal Ageing Study

Tze Pin Ng^{a, b} Xin Zhong^c Qi Gao^a Xinyi Gwee^a Denise Qian Ling Chua^a
Anis Larbi^{d, e}

^aDepartment of Psychological Medicine, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore; ^bGeriatric Education and Research Institute, Ministry of Health, Singapore, Singapore; ^cSocial and Cognitive Computing Department, Institute of High-Performance Computing, Agency for Science, Technology and Research (A*STAR), Fusionopolis, Singapore, Singapore; ^dSingapore Immunology Network (SigN), Agency for Science Technology and Research (A*STAR), Singapore, Singapore; ^eDepartment of Biology, Faculty of Sciences, University Tunis El Manar, Tunis, Tunisia

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Abstract

Introduction: The identification of modifiable health span-promoting factors is a public health priority. **Objective:** To explore the socio-environmental, lifestyle, behavioural, and psychological determinants of a clinical phenotypic measure of biological ageing in the Singapore Longitudinal Ageing Study (SLAS) cohort. **Methods:** Using cross-sectional data on 2,844 SLAS-2 adults with a chronological age (CA) ≥ 55 years, we estimated biological age (BA) using a validated panel of clinical, biochemical, physiological, and functional indicators (8 in men and 10 in women) and calculated the difference between BA and CA (BA – CA in years). Potential determinants included education, housing status, loss of a spouse, living alone, lifestyle and health activity, smoking, alcohol consumption, nutritional risks, consumption of milk, soy, fruit, vegetables, coffee and tea, sleep parameters, and life satisfaction. **Results:** The mean CA was 67.0 (standard deviation [SD] 7.9; range 55–94) years. The estimated BA varied more widely (SD 8.9 years; range 47.5–119.9 years), and

BA – CA ranged from –11.3 to 30.0 years. In stepwise selection regression analyses, multiple significant independent determinants in a final model were larger for private housing, being single/divorced/widowed, productivity, cognitive and leisure time activity scores, 10 h/week of moderate-to-vigorous physical activity, unintended loss of weight, life satisfaction, and daily consumption of fruits 1–2 or ≥ 3 servings and Chinese tea 1–2 or ≥ 3 cups daily, together explaining 16% of BA – CA variance in men and 14% in women. Associated BA – CA estimates were highest in men with high-end housing status (–1.8 years, effect size 0.015) and unintended weight loss (1.5 years, effect size 0.017). **Conclusion:** We identified determinants of biological ageing which can promote health span.

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Introduction

With the global population ageing, countries all over the world currently face the mounting societal challenges of dealing with the increasing burden of disease and disability resulting from a longer life expectancy. Much of the gains in life expectancy have thus far been achieved by

advances in medical care and public health [1] made in past decades that reduce the mortality associated with individual diseases, particularly infectious diseases, malnutrition, and cardiovascular disease, with the exception of diabetes. Currently, there is an axiomatic need for finding ways to help people stay healthy and free of disability as they age, i.e., extending a healthy life expectancy (“health span”). Given that the majority of the elderly have multiple diseases, it has been argued that the extent to which effective treatment or the prevention of individual diseases is able to improve health span is limited [2]. For this reason, it is proposed that the slowing of ageing itself is probably the most effective strategy for extending health span [3].

Research into the area of biological ageing and health span extension has accelerated at a phenomenal rate in the past decade. There is broad consensus that the molecular changes associated with ageing can be slowed or reversed in animals, resulting in an increased healthy life span. However, the clinical translation of these findings to health span extension in humans is lengthy and complex. Clinical studies investigating molecular targets (e.g., metformin, rapamycin analogues, etc.) of health span extension in humans are nascent [4]. These translational prospects are made possible by very recent important contributions to measuring biological ageing in humans.

Given that biological ageing reflects the progressive deterioration of biological system integrity at multiple (i.e., molecular, cellular, tissue, organ, and whole-body) levels, a number of approaches have been proposed to measure biological ageing. These measures of biological age (BA) broadly include cellular and molecular estimators such as “telomeric age” (based on leukocyte telomere length [5, 6]), DNA methylation age (“epigenetic clocks”) [7–12], physiological age (based on clinical measures and routine blood biomarkers), and functional age (cognitive age, functional age, and Frailty Index [FI] [13–16]). Studies have shown that all BA estimators correlate to varying degrees except for telomere length and are associated with mortality risk independently of chronological age (CA). Regarding different BA estimators, measures of physiological age and functional age, especially the FI, appear to be stronger and more robust predictors of mortality [17–21], although more recent algorithmic versions of DNA methylation age (DNAmGrimAge or Dunedin-PoAm) show similar large effects, and, jointly, DNA methylation and FI remain independent predictive of mortality [21]. Given the current state of science, it thus appears that molecular and physiological and/or functional estimators of BA reflect different ageing pheno-

types and may complement each other for application in different settings.

Clinical phenotypic measures of biological ageing based on composite measurements of standard clinical chemistry and physiological and functional measures of ageing are relatively inexpensive and simple to use, making them suitable for evaluating the effects of clinical or public health interventions aimed at extending health span. Several measures of BA have been created and validated in a number of different populations [13–16] including in Asia [22–24]. Recently, we developed a population-specific measure of biological ageing among Singaporean Chinese, based on routine blood chemistry and physiological and functional biomarkers (8 in men and 10 in women) that robustly differentiate biologically older and younger individuals with the same CA, with respect to their future risk of physical frailty and mortality [25]. For 2 individuals with the same CA, the one who is biologically older based on his/her estimated BA is more likely to become frail or die earlier, and vice versa.

While studies of clinical interventions to extend health span are progressing apace, it has also been recently highlighted that an urgent public health priority is the identification and amelioration of modifiable lifestyle, behavioural, and environmental factors that could help to increase health span [26]. Factors such as physical activity, nutrition, and stress avoidance are most likely the sort of modifiable determinants of healthy life span, but there is a paucity of studies that have investigated and documented some but not all social and behavioural factors [12, 15, 21, 27–29]. The aim of this study was to attempt a wide-ranging exploration of lifestyle, behavioural, psychological, and socio-environmental determinants of a validated clinical phenotypic measure of biological ageing that was recently reported in a previous study on the subjects and data from the Singapore Longitudinal Ageing Study (SLAS).

Methods

Study Population

The SLAS was a population-based prospective cohort study on the ageing and health of middle-aged and older adults (i.e., ≥55 years) in Singapore. Details of the SLAS are available in a previous publication [30]. The extensive range of baseline data collected from questionnaire interviews, clinical and performance-based testing and examinations, and blood sampling, included psychosocial, lifestyle, behavioural, medical, biological, physiological, dietary and nutritional, physical and neurocognitive functioning, and health status variables. This study analysed data on 2,844 Chinese adults aged 55–94 years in the second cohort (SLAS-2), who

Fig. 1. Correlations between Klemmler-Doubal (KM) estimates of biological age (BA) and chronological age (CA).

were recruited from March 2008 to November 2013. As reported in our previous publication, the clinical phenotypic measure, BA, was developed and validated through follow-up re-interviews and assessments of incident physical frailty at intervals of 4–5 years from January 2013 to August 2018, and of mortality up to December 2016. We analysed the baseline data on lifestyle, behavioural, psychological, and socio-environmental determinants and biological ageing in this cross-sectional study.

Clinical Phenotypic BA Measure

We used a sex-specific measure of BA derived from and validated in the SLAS cohort, as described previously [25]. The biomarkers were based on routine clinical measurements of blood, physiological, and functional biomarkers that are commonly available in clinical practice. They were selected from a total of 68 (32 blood or serum; 36 clinical and functional) different biomarkers by initially identifying those that were significantly associated with CA

($r \geq 0.2$, $p < 0.01$), and then removing those which were highly associated with other biomarkers but with a relatively small contribution to the estimation of age. Stepwise selection, multiple linear regression modelling resulted in the final selection of 8 markers for males, covering physiological functioning in 6 domains of vascular function (sitting diastolic blood pressure); lung function (FEV_1), physical function (handgrip strength, knee extension strength, timed up and go, and gait speed), haematological status (haemoglobin level), kidney function (estimated glomerular filtration rate [eGFR]), and cognition (Mini-Mental State Examination [MMSE] score). For females, 10 markers were selected that covered physiological functioning in 6 domains: body composition (body mass-height and thigh circumference), lung function (FEV_1), physical function (handgrip strength, knee extension strength, timed up and go, gait speed, and chair rise), haematological status (lymphocyte count), kidney function (eGFR), and cognition (MMSE score). The computation for estimating BA included the Klemmler-Doubal

Table 1. Chronological and biological age of 2,844 SLAS-2 Chinese cohort participants; demographic, socio-environmental, lifestyle, behavioural, psychological, and nutritional determinants

Chronological age, years	67.2±7.9 (55–94)
Biological age, years	67.2±8.9 (47.3–119.9)
BA – CA, years	0.0±4.12 (–11.7 to 30.0)
Female sex	62.6 (1,780)
Years in education, ≤6/7–10/≥11	21.3 (607)/41.8 (1,188)/36.5 (1,039)
Housing (low- to high-end) ^a	21.7 (616)/27.1 (772)/44.9 (1,278)/6.3 (178)
Single, divorced, or widowed	34.8 (990)
Live alone	16.4 (467)
Health activity score	9.87±1.99 (2–12)
Social activity score	3.06±2.67 (0–16)
Productive activity score	3.79±1.92 (0–8)
Physical and fitness activity score	2.19±1.53 (0–8)
Recreational activity	4.20±1.96 (0–8)
Cognitive activity score	1.45±0.85 (0–2)
Summary leisure time activity score	24.55±6.58 (5–52)
Hours of moderate-to-vigorous physical activity	11.0±4.45 (0–28)
Cigarette smoking ^b	76.9 (2,186)/8.4 (239)/4.2 (120)/8.5 (243)/2.0 (56)
Alcohol consumption (≥3 drinks daily)	9.5 (270)
Illness determines type or amount of food eaten	2.1 (59)
<2 meals eaten per day	30.7 (872)
Difficulty eating due to oral/dental problems	3.7 (105)
Not enough money to buy needed food	8.3 (234)
Eat alone most of the time	3.3 (94)
Take ≥5 different drugs per day	24.4 (693)
Unintended weight loss (4.5 kg in last 3 months)	18.1 (514)
Unintended weight gain (4.5 kg in last 3 months)	1.3 (36)
Am unable to shop/cook/feed myself	0.9 (27)
Nutritional risk score	1.74±2.20 (0–17)
Dietary intake ^c	
Dairy products	42.8 (1217)/10.1 (287)/14.9 (423)/3.1 (87)/29.2 (830)
Soy products	14.1 (399)/19.6 (558)/52.8 (1,502)/9.1 (260)/4.4 (125)
Deep-sea fish	25.4 (722)/23.2 (659)/38.4 (1,091)/7.7 (219)/5.4 (153)
Coastal-water fish	13.9 (395)/15.8 (449)/52.1 (1,482)/11.2 (318)/7.0 (200)
Freshwater fish	48.8 (1,389)/18.3 (520)/25.5 (725)/4.9 (139)/2.5 (71)
Seafood other than fish	50.2 (1,427)/28.8 (818)/18.8 (535)/1.8 (51)/0.5 (13)
Number of fruit servings per day (0, 1, 2, 3)	18.4 (524)/52.3 (1,488)/27.0 (768)/2.3 (64)
Number of vegetable servings per day (0, 1, 2, 3)	5.1 (144)/31.2 (886)/60.9 (1,733)/2.8 (81)
Intake of hot beverages ^d	
Coffee	21.5 (611)/2.7 (78)/5.1 (146)/63.5 (1,805)/6.5 (186)
Tea (all types)	31.0 (883)/15.0 (427)/13.7 (389)/4.2 (120)/2.5 (71)
Western tea	44.9 (1,278)/7.7 (218)/6.8 (192)/4.5 (127)/2.6 (75)
Chinese tea	41.8 (1,190)/8.6 (245)/7.7 (220)/5.1 (146)/3.1 (89)
Green tea	56.7 (1,612)/4.3 (122)/3.2 (90)/1.3 (37)/1.0 (29)
Sleep disturbances ^e	
Difficulty getting to sleep	56.6 (1,611)/15.0 (426)/12.5 (356)/7.7 (220)/7.7 (218)
Difficulty staying asleep	55.2 (1,571)/18.4 (522)/12.8 (365)/6.6 (189)/6.5 (186)
Early morning waking	57.9 (1,647)/18.6 (529)/12.8 (365)/5.7 (162)/4.7 (135)
Life satisfaction summed score	16.30±2.39 (6–20)
Life is boring...interesting ^f	3.93±0.67 (1–5)
Life is sad...happy ^f	3.94±0.67 (1–5)
Life is easy...hard ^f	3.92±0.69 (1–5)
Life is lonely...not lonely ^g	2.83±0.42 (1–3)

Values are expressed as % (n) or mean ± standard deviation (min–max score).

^a 1- or 2-room public housing/3-room public housing/4- or 5-room public housing/private condominiums/landed houses.

^b Never-smoker/ex-smoker <20 per day/ex-smoker ≥20 per day/current <20 per day/current ≥20 per day.

^c Never or rarely/more than once per week/1–3 times per week/4–6 times/week/daily.

^d Never or rarely/<1 cup per week/≥1 cup per week to <1 cup daily/1–2 cups daily/≥3 cups daily.

^e Never/rarely (<1× per month)/sometimes (≥1× per month <1× per week)/frequently (≥1× per week, not daily)/daily.

^f A scale of 1–5, where 1 = very, 2 = fairly, 3 = neither, 4 = fairly, and 5 = very.

^g A scale of 1–3, where 1 = very, 2 = fairly, and 3 = not at all.

(KD) algorithm, which was found to be more optimal than Principal Component Analysis, Multiple Linear Regression, and Machine Learning methods for predicting mortality and frailty. The KD estimator of BA was found to be significantly better than CA in predicting mortality and frailty (C-statistics ranging from 0.70 to 0.79 in the middle-aged group), thus supporting its relevance and usefulness for measuring health span in middle-aged individuals. The estimated BA for each individual varied by as much as 10–30 years above and below the mean (2SD) for each value of CA from 55 to 94 years. In this study, we used the residual index, BA – CA, to denote whether a person was biologically younger (a negative value in years) or older (a positive value in years) than their CA (Fig. 1).

Determinants of BA

Socio-Environmental Variables. These included measures of socio-economic status: years in education (≤ 6 , 7–10, ≥ 11 years), housing status (lower- to higher-end, i.e., public housing (1 or 2, 3, and 4 or 5 rooms, a private condominium or apartments, and property with land), and measures of social isolation (single/divorced/widowed and living alone).

Health and Lifestyle Activities. Health activity was measured using a summed Likert score of frequency of engagement, i.e., 1 = never (less than once a month); 2 = sometimes (once a month or more but less than once a week); 3 = often (once a week or more), in health-related activities, namely, watch what you eat, exercise regularly (i.e., 2–3 times a week), good sleep habits, make time for leisure and relaxation, and habits regarding smoking and alcohol consumption.

Lifestyle and Leisure Time Activity. These were measured for 8 categories of social activities (attending religious worship, sport events, excursions, card games, seniors' clubs, and group singing or dancing), 5 categories of productive activities (hobbies, cookery, shopping, volunteerism, and paid or unpaid employment), 3 categories of physical and fitness activities (brisk walking, exercise routines, and sports), recreational activities (television, radio, music, computer games), and 1 category of cognitively stimulating activities (reading, drawing, painting, photography, attending courses, puzzles, mind games, etc.). Summed Likert scores of the frequency of participation (as above) for each domain of social, productive, physical and fitness, recreational, and cognitive activities and a total summary score of health and lifestyle activities was computed. Additionally, the estimated total number of hours of participation in moderate- and vigorous-level physical activities was reported by the respondents.

Sleep Health. Respondents reported the frequency (1 = never; 2 = rarely, i.e., $< 1 \times / \text{month}$; 3 = sometimes, i.e., $\geq 1 \times / \text{month}$ but $< 1 \times / \text{week}$; 4 = frequently, i.e., $\geq 1 \times / \text{week}$ but not daily, 5 = always, i.e., daily, of disturbed sleep (difficulty getting to sleep or staying asleep and early-morning waking).

Life Satisfaction. This was measured using Likert response scores to 3 questions on whether the respondent finds his/her life to be boring or interesting, sad or happy, hard or easy on a scale of 1–5 (1 = very boring, 2 = fairly boring, 3 = neither, 4 = fairly interesting, 5 = very interesting) and 1 question on whether the respondent felt lonely (1 = very lonely, 2 = fairly lonely, 3 = not at all lonely). A summed score of total life satisfaction was derived.

Smoking. Participants reported their smoking history and were categorized as 1 = never-smokers, 2 = ex-smoker of < 20 cigarettes/day, 3 = ex-smoker of ≥ 20 cigarettes/day, 4 = current smoker of < 20 cigarettes/day, 5 = current smoker of ≥ 20 cigarettes/day.

Alcohol. Self-reported history of alcohol consumption was used to categorize participants by their level of drinking, i.e., ≥ 3 drinks a day (= 1) or less (= 0).

Nutritional Risk. We used the DETERMINE Your Nutritional Health check-list, developed for the Nutrition Screening Initiative by the American Dietetic Association, the American Academy of Family Physicians, and the National Council on Ageing in the USA, to assess the risk of malnutrition in older adults. The check-list includes 10 Yes/No question-items describing personal and behavioural factors related to inadequate or poor-quality food and nutrient intake among older people (Table 1). Weighted scores for individual responses are summed to give a total score ranging from 0 to 21; a score of ≥ 6 indicates a high nutritional risk, 3–5 a moderate nutritional risk, and 0–2 a good nutritional status.

Additionally, participants gave their responses on the frequency of specific intake of milk and dairy products, soy products, fish (deep-water, coastal, fresh-water, and other seafood): 1 = never or rarely, 2 = $< \text{once/week}$, 3 = 1–3 times/week, 4 = 4–6 times/week, 5 = daily. Consumption of fruit and vegetables was quantitated by the number of servings (0, 1, or 2+) consumed per day. The frequency of coffee and tea consumption (Chinese black and oolong tea, green tea, and Western black tea drunk with milk) was quantitated as 1 = never or rarely, 2 = $< 1 \text{ cup/week}$, 3 = $\geq 1 \text{ cup/week}$ to $< 1 \text{ cup/day}$, 4 = 1–2 cups/day or 5 = $\geq \text{cups/day}$, and the equivalent frequency and amount of tea of all types was computed.

Disease and Disability. The presence and number of chronic diseases in individual participants were ascertained by self-reports of a physician diagnosis and treatment for 18 common diagnoses and others in the questionnaire, corroborated by the recording of names of drugs on medication packages produced by the participants as well as measurements of their blood pressure, fasting blood glucose, lipids, and eGFR. For example, hypertension included known cases of anti-hypertensive treatment as well as unknown or untreated cases with raised systolic or diastolic blood pressure based on standard clinical criteria. Raised fasting blood glucose was used to define prediabetes or undiagnosed diabetes, and eGFR $< 60 \text{ mL/min/1.73 m}^2$ to define chronic kidney disease, according to standard clinical criteria. The number of comorbidities was estimated from the total count of medical disorders in the past year. Disability was measured by dependency when performing the basic and instrumental activities of daily living used in the Barthel and the Lawton indexes.

Statistical Analysis

We used regression analyses to estimate the associations of individual determinant variables with residual BA – CA as the normally distributed continuous dependent variable. The total of 49 potential determinants included untransformed continuous variables that met assumption of normal distribution and were tested for linear relationships with BA – CA, and ordinal variables that were analysed categorically with ANOVA testing for non-linear relationships (online supplementary Tables S1 and S2; for all online suppl. material, see www.karger.com/doi/10.1159/000511211). Mean BA – CA estimates from multiple group comparisons were made with the Bonferroni correction (95% confidence interval [CI]). A forward stepwise selection approach was used to identify significant determinants in a final model of multiple independent determinants that are significant in the presence of each other using probability at entry of $p < 0.05$ and at removal of $p < 0.10$. The results were presented separately for men and women in stratified analyses by sex.

Table 2. Regression estimates of BA – CA associated with significant determinants in men and women

Significant determinants	Men				partial η^2	Women				partial η^2
	mean difference (95% CI)			<i>p</i> overall		mean difference (95% CI)			<i>p</i> overall	
Education: 1 = ≤6 years	0				0.036	0				0.035
2 = 7–10 years	–1.165	–2.135	–0.194	*		–1.158	–1.561	–0.756	***	
3 = ≥11 years	–2.657	–3.623	–1.690	***		–1.729	–2.156	–1.303	***	
Housing: 1 = 1 or 2 rooms public	0				0.083	0				0.042
2 = 3 rooms public	–1.533	–2.329	–0.740	***		–1.336	–1.818	–0.855	***	
3 = 4 or 5 rooms public	–3.303	–3.743	–2.322	***		–1.837	–2.278	–1.395	***	
4 = private housing	–4.489	–5.664	–3.314	***		–2.578	–3.364	–1.791	***	
Single/divorced/widowed	2.798	2.139	3.457	***	0.061	0.444	0.106	0.781	**	0.004
Live alone	1.143	0.346	1.941	**	0.007	0.297	–0.151	0.744		0.001
Health and lifestyle activity										
Health activity, per unit score	–0.224	–0.359	–0.089	***	0.010	–0.212	–0.302	–0.122	***	0.012
Social activity, per unit score	–0.160	–0.281	–0.039	**	0.006	–0.198	–0.257	–0.138	***	0.024
Productive activity, per unit score	–0.532	–0.681	–0.383	***	0.045	–0.343	–0.437	–0.249	***	0.029
Physical and fitness activity, per unit score	–0.677	–0.868	–0.486	***	0.044	–0.396	–0.503	–0.289	***	0.029
Recreational activity, per unit score	–0.471	–0.615	–0.328	***	0.038	–0.233	–0.319	–0.147	***	0.016
Cognitive activity, per unit score	–1.453	–1.844	–1.062	***	0.048	–0.787	–0.969	–0.606	***	0.039
Summary leisure time activity, per unit score	–0.192	–0.237	–0.147	***	0.066	–0.123	–0.147	–0.098	***	0.055
Hours/week of moderate-to-vigorous physical activity	–0.126	–0.193	–0.059	***	0.014	–0.118	–0.156	–0.080	***	0.022
Life is very boring...very interesting (1–5)	–1.148	–1.580	–0.716	***	0.025	–0.572	–0.822	–0.321	***	0.011
Life is very sad...very happy (1–5)	–1.170	–1.594	–0.745	***	0.027	–0.497	–0.747	–0.247	***	0.009
Life is very hard...very easy (1–5)	–0.896	–1.306	–0.486	***	0.017	–0.427	–0.672	–0.183	***	0.007
Life is very lonely...not at all (1–3)	–1.295	–1.964	–0.626	***	0.014	–0.487	–0.896	–0.079	*	0.003
Life satisfaction total score (6–20)	–0.448	–0.592	–0.304	***	0.034	–0.189	–0.273	–0.105	***	0.011
Smoking										
Never-smoker	0					0				
Ex-smoker	0.351	–0.342	1.044			–0.265	–.231	0.700		
Current smoker	1.089	0.333	1.845	**	0.007	1.438	0.548	2.327	**	0.006
Alcohol consumption, ≥3 drinks/day	1.344	0.068	2.619	*	0.004	–0.354	–2.113	1.406		0.000
Not enough money to buy needed food	2.370	1.396	3.344	***	0.021	0.123	–0.522	0.768		0.000
Eat alone most of the time	2.670	1.253	4.087	***	0.013	1.518	0.475	2.562	***	0.005
Take ≥5 different drugs/day	1.442	0.725	2.158	***	0.014	0.773	0.397	1.149	***	0.009
Unintended weight loss (>4.5 kg in last 3 months)	1.919	1.187	2.651	***	0.024	1.898	1.465	2.331	***	0.040
Not able to shop/cook/feed myself	0.473	–2.453	3.400		0.000	3.938	2.190	5.687	***	0.011
Nutritional risk per point score	0.460	0.335	0.584	***	0.047	0.177	0.099	0.255	***	0.011
Soy products										
Never or rarely	0				0.019	0				0.013
More than once/month and <once/week	–0.037	–1.060	0.986			–0.142	–0.720	0.436		
1–3 times a week	–0.787	–1.669	0.095			–0.786	–1.282	–0.291	**	
4–6 times a week	–1.832	–3.061	–0.603	**		–0.912	–1.620	–0.204	*	
Daily	–2.569	–4.111	–1.028	**		–1.698	–2.625	–0.772	***	
Deep-sea fish										
Never or rarely	0				0.009	0				0.007
More than once/month and <once/week	–0.593	–1.417	0.227			–0.680	–1.164	–0.195	**	
1–3 times a week	–0.872	–1.625	–0.120	*		–0.713	–1.138	–0.654	**	
4–6 times a week	–1.631	–2.881	–0.381	**		–0.654	–1.324	0.016		
Daily	–1.411	–2.888	0.066			–0.855	–1.619	–0.091	*	
Fruit servings per day										
0	0				0.023					0.023
1	–1.585	–2.33	–0.841	***		–1.012	–1.477	–0.547	***	
2	–2.073	–2.924	–1.221	***		–1.637	–2.137	–1.136	***	
Vegetable servings per day										
0	0				0.008	0				0.004
1	–1.626	–3.000	–0.252	*		–1.162	–2.318	–0.007	*	
2	–1.911	–3.240	–0.582	**		–1.438	–2.576	–0.301	*	
Tea (all types)										
Never or rarely	0				0.008	0				0.023
<1 cup a day	–0.205	–1.083	0.673			–0.823	–1.304	–0.343	***	
≥1 cups a day	–1.095	–2.013	–0.178	**		–1.275	–1.981	–0.569	***	
Chinese tea										
Never or rarely	0				0.006	0				0.017
<1 cup a day	–0.484	–1.373	0.404	*		–0.653	–1.500	0.193		
≥1 cups a day	–1.023	–2.032	–0.014			–2.502	–3.665	–1.339	***	

Table 3. Significant lifestyle variables in final stepwise selection model of BA – CA years in men and women

	Men				Women			
	difference	95% CI	<i>p</i> value	η^2	difference	95% CI	<i>p</i> value	η^2
Housing type (vs. 1- or 2-room public housing)				0.015				0.009
3-room public housing	–0.206	–1.089 to 0.677			–0.401	–1.003 to 0.202		
4–5-room public housing	–1.256	–2.122 to –0.400	**		–0.836	–1.408 to –0.264	**	
Private condominiums and landed housing	–1.827	–3.197 to –0.457	**		–1.101	–2.020 to –0.181	*	
Single/divorced/widowed	1.390	0.601 to 2.179	***	0.013	ns	ns		ns
Life satisfaction (interesting)	–0.551	–0.999 to –0.103	*	0.006	ns	ns		ns
Productive activity, per point score	–0.219	–0.403 to –0.034	*	0.006	ns	ns		
Cognitive activity, per point score	–0.734	–0.285 to –0.011	**	0.011	–0.304	–0.559 to –0.050	*	0.005
Leisure time activity total score	–0.065	–0.127 to –0.003	*	0.005	–0.089	–0.124 to –0.005	***	0.023
Hours/week moderate-to-vigorous physical activity	–0.068	–0.133 to –0.002	*	0.004	–0.071	–0.118 to –0.025	**	0.008
Unintended weight loss (>4.5 kg in last 3 months)	1.510	0.765 to 2.254	***	0.017	1.160	0.600 to 1.719	***	0.014
Fruit servings (vs. 0–1 daily)								0.014
2 daily	ns	ns		ns	–0.729	–1.305 to –0.154	*	
3 daily	ns	ns		ns	–1.265	–1.896 to –0.633	***	
Chinese tea (vs. never or rarely)								0.008
<1 cup/day	ns	ns		ns	–0.466	–0.930 to 0.003	*	
≥1 cups/day	ns	ns		ns	–0.879	–1.565 to –0.194	**	
Adjusted R^2				0.161				0.142

Results

The mean CA of the cohort was 67.0 (SD 7.9; range 55–94) years. The estimated BA varied more widely (SD 8.9 years), ranging from 47.5 to 119.9 years, and was higher for men than women (mean 67.8 [SD 9.3] and 66.7 [SD 8.7], respectively). As indicated by the BA – CA residuals, the BA for individuals varied by as much as 11 years below and 30 years above the CA (Table 1; Fig. 1). There was no difference in BA – CA years between men and women.

Estimates of the association of the BA – CA residuals with all 49 possible determinants as shown by the mean differences and 95% CI of BA – CA in years between groups or per point scores are listed in online supplementary Tables S1 and S2. Determinants which showed statistically significant ($p < 0.05$) associations with BA – CA years in men and women are shown in Table 2. For example, more years in education (7–10 years and ≥ 11 years relative to ≤ 6 years) were associated significantly ($p < 0.001$) with BA – CA estimates of –1.16 and –2.66 years, respectively, in men, and –1.16 and –1.73 years, respectively, in women, suggesting that more highly educated individuals are more likely to be biologically younger by 1–2 years. Other variables that were significantly associated with a younger BA were higher levels of education

and better housing, health and lifestyle activities, life satisfaction, and a diet including the consumption of soy products, deep-sea fish, fruit and vegetables, and tea. Variables that were significantly associated with older BA were being single, divorced, or widowed, living alone, smoking, alcohol consumption, having a higher nutritional risk, and a poor nutritional status.

In the stepwise selection regression analyses, independent determinants in a final model which remained significant in the presence of each other were: better-quality housing, being single, divorced, or widowed, and life satisfaction (in men); and being productive, engaging in cognitive activity, leisure activities, the number of hours per week of moderate-to-vigorous physical activity, unintended loss of weight, daily fruit consumption, and drinking Chinese tea (in women) (Table 3).

The estimates of BA – CA associated with these significant variables of interest were further adjusted for the presence of disability and the number of co-occurring chronic diseases as forced-entry covariates in expanded models. The adjusted BA – CA estimates associated with lifestyle, behavioural, and socio-environmental variables of interest were attenuated when controlled for the presence of disease and disability, but basically remained significantly associated with BA – CA (Table 4).

Table 4. Significant lifestyle variables in final stepwise selection model of BA – CA years in men and women, adjusted additionally for number of comorbidities and disability

	Men				Women			
	difference	95% CI	<i>p</i> value	η^2	difference	95% CI	<i>p</i> value	η^2
Housing type (vs. 1- or 2-room public housing)				0.007				0.011
3-room public housing	–0.554	–1.395 to 0.287			–0.441	–1.036 to 0.154		
4–5-room public housing	–1.596	–2.412 to –0.780	***		–0.883	–1.446 to –0.320	**	
Private condominium and landed housing	–2.207	–3.472 to –0.941	***		–1.135	–2.044 to –0.225	*	
Single/divorced/widowed	1.348	0.597 to 2.100	***	0.012	ns	ns		ns
Life satisfaction (interesting)	–0.455	–0.880 to –0.029	*	0.004	ns	ns		ns
Productive activity, per point score	–0.249	–0.401 to –0.097	*	0.010	ns	ns		
Cognitive activity, per point score	–0.777	–1.181 to –0.373	***	0.014	ns	ns		0.005
Leisure time activity total score	ns	ns		ns	–0.090	–0.122 to –0.058	***	0.027
Hours/week moderate-to-vigorous physical activity	ns	ns		ns	–0.052	–0.099 to –0.005	*	0.004
Unintended weight loss (>4.5 kg in last 3 months)	1.159	0.324 to 1.993	**	0.017	0.773	0.142 to 1.403	*	0.005
Fruit servings (vs. 0–1 daily)								0.015
2 daily	ns	ns		ns	–0.795	–1.367 to –0.224	**	
3 daily	ns	ns		ns	–1.285	–1.912 to –0.659	***	
Chinese tea (vs. never or rarely)								0.007
<1 cup/day	ns	ns		ns	–0.393	–0.855 to 0.069		
≥1 cups/day	ns	ns		ns	–0.852	–1.532 to –0.172	**	
Adjusted <i>R</i> ²				0.148				0.159

The effect sizes estimated by the partial η^2 were small for individual determinants and were highest for housing status (1.5%) and unintended weight loss (1.7%). The final model of all significant determinants of BA – CA had an *R*² value of 16% in men and 14% in women, suggesting remaining unexplained variances in BA – CA due to unmeasured factors.

We estimated the combined effects of significant determinants of BA in the individual by creating a Health Span Factors Score (HSFS), i.e., a summed score of binary and ordinal integer scores (0, 1, and 2) for each of the significant predictors, with higher scores denoting a positive valence value of health span, except for weight loss which was assigned a negative value of –1. The mean HSFS of the participants was 8.0 (SD 2.9; range 1–15). Figure 2 shows the mean and 95% CI of BA – CA in years associated with HSFS, showing a linear declining trend from 3.5 years (2.5–4.6) for an HSFS of 1 to –2.5 years (–3.9 to –1.1) for an HSFS of 15.

Discussion

To achieve the goal of enhancing the well-being of individuals as they age, the factors that determine the trajectories of ageing need to be better understood. Our

study reveals a larger number of lifestyle, behavioural, and socio-environmental factors are linked to biological ageing than previously thought, and provides vital insights into potentially modifiable determinants of healthy life span for targeted interventions. In retrospect, the results are probably not surprising, as the same health risk and protective factors are also known determinants of chronic diseases and disability.

The clinical phenotypic measure of biological ageing used in this study represents organ-system level physiological and functional manifestations of multiple cellular and molecular processes of ageing. The variations in biological ageing, as such, that are explained by health risk variables are posited to be mediated by cellular processes of biological ageing like epigenetic modifications, telomere erosion, mitochondrial energy balance dysregulation, and inflammation. There are cellular-level measures of biological ageing such as epigenetic, telomere, mitochondrial DNA, inflammation, and “omics” “clocks” that we did not use in this study. Currently, there is still much to understand about the inter-relationships of clinical and molecular measures of BA, whether they measure the same or different processes of biological ageing, and their causal roles in the ageing process and diseases. Recent studies suggest that the correlations between extant clinical

Fig. 2. Mean and 95% CI estimates of BA – CA in years for integer values of Health Span Factors Score (HSFS). Linear regression: BA – CA $3.819 - 0.476 \times \text{HSF}$. HSFS is the summed score of education (0, 1, 2), housing status (0, 1, 2, 3), productive activity (0, 1, 2), physical and fitness activity (0, 1, 2), cognitive activity (0, 1), ≥ 10 h/week of moderate to vigorous activities (0, 1), daily fruit servings (0, 1, 2), tea consumption (0, 1, 2), life very interesting (0, 1), and loss of weight (0, –1). Cutoff values are as shown in the Tables. Productive activity cutoffs were 0–2, 3–4, 5–10; physical and fitness activity cutoffs were 0–1, 2, and 3–8.

cal biomarkers and cellular or molecular biomarker measures of ageing (in particular, the epigenetic clock and telomere length) are, in general, weak, suggesting that they quantify different processes of biological ageing [17–21].

Our study adds to a number of recent studies, exploring the socio-environmental and health-related determinants of health span, that have used molecular or composite physiological clinical biomarkers of biological ageing. These studies also reported similar relationships with summary measures of life-course risk factors including low educational attainment, material and social resource deficits, and mental health problems [12, 15, 19, 21, 27–29]. Notably, an early personal and childhood history of familial longevity, social class, adverse experiences, and health, intelligence, and self-control were shown to predict more rapid physiological ageing (using the Pace of Ageing Index) [27]. A low level of education and smoking, relatively more than other lifestyle-related risk factors such as alcohol intake or obesity, were also shown to be associated with accelerated epigenetic ageing [28]. In another recent study, health-related lifestyle behaviours (obesity, smoking, alcohol consumption, and physical activity) exhibited the greatest contribution to the Pheno-

Age Index of biological ageing, closely followed by adversity in adulthood [29].

In line with previous studies, our study provides further evidence in support of the predominant influence of socio-environmental circumstances and lifestyle behavioural factors on biological ageing. Socio-economic factors reflecting access to material and social resources appeared as the strongest determinants of health span, and physical and cognitive activities stand out as amenable behavioural factors. In addition, our study revealed other lifestyle behavioural factors such as the consumption of fruit and tea, which have not been reported in previous studies. Their estimated associations are robust to the statistical control of mutual confounding by multiple covariates in the models and also consistent with established knowledge about their influence on health and disease. For example, tea which is rich in polyphenols such as epigallocatechin-3-gallate (EGCG) with powerful antioxidant and anti-inflammatory actions, is regarded as an “anti-senescence food” for healthy ageing [31, 32].

The effect sizes associated with individual determinants were small but can, collectively, contribute substantially to extending health span in real-world settings. The HSFS of the individual, reflecting the number and intensity of health span-promoting factors, suggest that, for those in the highest-percentile group, BA may be younger than CA by ≥ 2 years at the population level (and some individuals may even be ≥ 10 years younger). Interestingly, this is consistent with the estimate that delaying ageing could increase population life expectancy by an additional 2.2 years, most of which would be spent in good health [2].

The effect size estimates of these determinants may be underestimated because of technical limitations in their measurements, which were derived from self-reports. Another limitation is that our BA estimator derived from the SLAS-2 cohort have yet to be further validated externally in an independent replication cohort. Because of the cross-sectional design of the study, the presence of prevalence or survival bias and the inability to fully capture exposure over a lifetime should be considered. Disentangling the relations between disease, disability, and biological ageing is difficult. Although health risk determinants were mostly found to remain significantly associated with the BA – CA measure of biological ageing after controlling for the number of comorbid chronic diseases and disability, cross-sectional analysis cannot establish causal relationships. Few longitudinal studies have investigated whether accelerated biological ageing

resulted from pre-existing diseases, or if the biological ageing was a risk factor prior to midlife that preceded disease onset. One study on young adults, the Dunedin birth cohort study, demonstrated that individuals showing rapid biological ageing before midlife were more susceptible to the effects of disease and physical and cognitive decline [14].

Other potential determinants of health span that were not examined in this study should be investigated in future studies. Another limitation is that the study population consisted of middle-aged and older adults, thus excluding younger adults who should also be targeted for preventive interventions to promote healthy ageing. Further studies should also aim at combining person-level measures with cellular-level measures of biological ageing to better understand the underlying biological mechanisms for extending health span. Finally, interventional studies using these surrogate measures of biological ageing are needed to demonstrate the efficacy of lifestyle behavioural interventions in extending human health span.

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Statement of Ethics

The research complied with the guidelines for human studies and it was conducted ethically in accordance with the Human Biomedical Research Acts (HBRA) of Singapore. All study participants gave their written informed consent and the study was approved by the National University of Singapore Institutional Review Board.

Conflict of Interest Statement

All authors declare there are no real or potential conflicts of interest. The sponsors had no role in the conduct of the study or preparation of this manuscript.

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Author Contributions

T.P.N. had full access to all of the data in the study and has primary responsibility for final content. T.P.N. reviewed the literature, designed the study, drafted and revised the manuscript. X.Z. and T.P.N. analysed the data. X.Z., Q.G., X.G., D.Q.L.C., and A.L. contributed to the study design and data collection. All authors reviewed the results and drafts, and approved the final manuscript.

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