Chronic inflammation as a determinant of future aging phenotypes.

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Chronic inflammation as a determinant of future aging phenotypes

Short Running Title: Inflammation and healthy aging

Tasnime N. Akbaraly, PhD[1], Mark Hamer, PhD[2], Jane E. Ferrie, PhD[3], Gordon Lowe, PhD [4], G. David Batty, PhD[5], Gareth Hagger-Johnson, PhD [2], Archana Singh-Manoux, PhD[6], Martin J. Shipley, MSc[2], Mika Kivimaki, PhD[2]

[1] Inserm U 1061, Montpellier F-34000, France

[2] Department of Epidemiology and Public Health, University College London, London, United Kingdom

[3]School of Social and Community Medicine, University of Bristol, UK

[4] Institute of Cardiovascular and Medical Sciences, University of Glasgow, the United Kingdom.

[5]Centre for Cognitive Ageing and Cognitive Epidemiology, University of Edinburgh, UK

[6] INSERM, U1018, Assistance Publique-Hopitaux de Paris, Paris, France

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Corresponding author: Tasnime Akbaraly INSERM U 1061, Hôpital La Colombière, 39 avenue Charles Flahault, BP 34493, 34093 Montpellier cedex 05, France.

Tel +33(0) 499 614 694 Fax : +33 (0) 499 614 579

Email: tasnime.akbaraly@inserm.fr

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Authors' contribution

TNA contributed to conception and design of study, conducted the statistical analyses, interpreted data, wrote the manuscript and is the guarantor. GL contributed to the acquisition of data and reviewed/edited the manuscript. MH, JEF, DB, GHJ, ASM contributed to conception and design of study, acquisition of data and reviewed/edited the manuscript. MJS contributed to conception and design of study, acquisition of data, statistical analyses and reviewed/edited the manuscript. MK contributed to conception and design of study, acquisition of data, contributed to the analysis and interpretation of the data. All of the authors approved the final version of the manuscript submitted for publication.

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ABSTRACT (250)

Background: The importance of chronic inflammation for aging phenotypes may have been underestimated in previous studies which use a single measurement of inflammatory markers. By assessing inflammatory markers twice over a 5-year exposure period we examine the association between long-term inflammation and future aging phenotypes in a large British population of men and women.

Methods: Data were drawn from 3044 middle-aged participants from the Whitehall II prospective study (28.2% women) with no history of stroke, myocardial infarction or cancer at baseline (1997-99). Interleukin-6 was assessed at baseline and 5 years before. Cause-specific mortality, chronic disease, and functioning were ascertained from hospital data, register linkage, and clinical examinations. We used these data to create 4 outcomes at the 10-year follow-up in 2007-09: successful aging (free of major chronic disease and with optimal physical, mental, and cognitive functioning, n=721), incident fatal or non-fatal cardiovascular disease (n=321), non-cardiovascular death (n=147), and normal aging (all others, n=1855).

Results: After adjustment for potential confounders, having high levels (>2 pg/mL) of interleukin-6 twice over the 5-year exposure period, nearly halved the odds of successful aging (odds ratio (OR) 0.53, 95% Confidence Interval (CI) 0.38–0.74) and increased risk of future cardiovascular events (OR=1.64, 95% CI 1.15–2.33) and non-cardiovascular death (OR= 2.43, 95% CI: 1.58–3.80).

Interpretation: If confirmed, these results shed new light on the importance of assessing long-term chronic inflammation by repeat measurements of interleukin-6 in geriatric clinical practice to target individuals at risk to develop unhealthy aging but also to promote ideal health.

INTRODUCTION

Chronic inflammation has been implicated in the etiology of age-related conditions(1), such as type 2 diabetes(2, 3), cardiovascular disease(4), cognitive impairment(5), and brain atrophy(6). Chronic inflammation may result from or be a cause of age-related disease processes (illustrated in Appendix 1). For example, obesity increases inflammation, and chronic inflammation, in turn, contributes to the development of type 2 diabetes, by inducing insulin resistance(7, 8), and to coronary heart disease, via atherogenesis(9). Thus, raised levels of inflammation appear to be implicated in various pathological processes leading to manifest disease in older age.

Of the various markers of systemic inflammation, interleukin-6 is particularly relevant to aging outcomes. There is increasing evidence that interleukin-6 is the pro-inflammatory cytokine which "drives" downstream inflammatory markers, such as C-reactive protein and fibrinogen(10, 11). Interleukin-6, in contrast to C-reactive protein and fibrinogen, is also likely to play a causal role in aging due to its direct effects on brain and skeletal muscles(12, 13). In addition, Mendelian randomisation studies of interleukin-6 and studies of antagonists are consistent with a causal role for interleukin-6 in relation to coronary heart disease, again in contrast to C-reactive protein or fibrinogen(14).

However, current understanding of the link between chronic inflammation and aging phenotypes is hampered by the methodological limitations of many existing studies. Importantly, most studies report an assessment of inflammation at one point in time, precluding a distinction between the short (acute) and longer-term (chronic) impact of the inflammatory process on disease outcomes(7). We address this issue using two measurements of interleukin-6, an average of 5 years apart to examine the association between chronic inflammation and a range of aging

phenotypes, assessed approximately 10 years later in a large British population of men and women. As inflammation characterises a wide range of pathological processes, we considered several aging phenotypes, including cardiovascular disease (fatal and non-fatal), non-cardiovascular mortality and successful aging which encompasses optimal functioning across different physical, mental, and cognitive domains.

METHODS

Study population

Data are drawn from the Whitehall II cohort, established in 1985 among 10,308 (67% men) British civil servants(15). All civil servants aged 35–55 years in 20 London-based departments were invited to participate; 73% agreed. The study consists of a nurse-administered clinical examination approximately every 5 years: 1985-88, 1991-93, 1997-99, 2002-04, and 2007-09. The study was approved by the University College London Ethics Committee and all participants gave a written consent.

To allow follow-up beyond age 60, the present analyses included participants aged 49 years and older in 1997-99, baseline for the current analyses. Analyses were restricted to the 3044 participants with no history of stroke, myocardial infarction, or cancer at baseline and for whom complete data on aging phenotypes in 2007-09 together with inflammatory markers assessed at baseline and 5 years before baseline (1991-93) and main baseline covariates were available. A flow chart depicting participant selection is shown in Figure 1.

Assessment of inflammatory markers

Interleukin-6, a marker of systemic inflammation, was measured using a high-sensitivity Enzyme-linked immunosorbent assay (R&D Systems, Oxford, United Kingdom) (16). At both 1991-93 and 1997-99 clinical examinations, blood samples were collected between 8 am and 1 pm, stored at -80 C and were not thawed or refrozen during storage. Stored serum samples from both phases were analysed in the same laboratory. Values below the detection limit (0.08 pg/mL for interleukin-6) were assigned a value equal to half the detection limit. Coefficients of variation

were 7.5 %. The reliability across follow-ups, assessed with Spearman's correlation coefficients, was 0.46.

C-reactive protein was also measured, using a high-sensitivity immunonephelometric assay in a Bn ProSpec nephelometer (Dade Behring, Milton Keynes, United Kingdom)(16).

Baseline covariates

Socio-demographic variables consist of sex, age and a 3-level measure of socioeconomic status (low, intermediate or high) based on Civil Service employment grade (15). Health behaviors considered in the analyses were: smoking status (never/former/current) and physical activity (inactive/moderately active/active) assessed by 20 questionnaire items on frequency and duration of participation in different activities as detailed elsewhere(17). Assessment of health status consisted of the use of anti-inflammatory medication, determined from details provided by participants regarding their medication use (generic name, brand name, or both). Possible cases of acute inflammation and immune activation due to current illness were also considered and defined as having C-reactive protein values >10 mg/L.

Outcome measures

Four aging phenotypes were considered(17): (1) successful aging (free of major chronic disease and with optimal physical, mental, and cognitive functioning); (2) non-fatal (clinically verified coronary heart diseases including myocardial infarction definite angina and self-reported stroke) or fatal cardiovascular disease event at follow-up; (3) non-cardiovascular mortality. Those who did not belong to any of these categories were categorized as (4) normal aging group. These health outcomes were ascertained using data from 2 follow-up screenings (2002-04; 2007-09)

plus records from national health registers. Further information on the categorization of aging phenotypes is provided in the Appendix 2.

Statistical Analysis

In these analyses the distributions of interleukin-6 both 5-y before baseline and at baseline (1997-99) were divided into three categories(18): low (≤1.0pg/mL, N=756), intermediate (1.1-2.0pg/mL, N=1456) and high (>2.0pg/mL, N=832). The number of times (0, 1 or 2) the participant had a high level of interleukin-6 (>2.0pg/mL) over the 5-year exposure period was the measure used to assess chronic inflammation(18).

Logistic regression models were used to assess the association of baseline interleukin-6 levels and over the 5-year exposure (measured 5-y before baseline and at baseline) with each aging outcome coded as a dichotomous variable: successful aging (versus normal aging phenotype, cardiovascular events and non-cardiovascular death combined), cardiovascular disease events (versus successful and normal aging phenotypes combined) and non-cardiovascular death (versus successful and normal aging phenotypes combined). Analyses were first adjusted for sex and age, and further adjusted for socio-economic status, health behaviors, acute inflammation and use of anti-inflammatory drugs. These analyses were repeated using C-reactive protein levels as the marker of inflammation. Further logistic regression models were performed specifically to examine the association between levels of interleukin-6 and each component of successful aging (good cardiovascular, respiratory, musculoskeletal, cognitive and mental well-being functioning and absence of diabetes, cancer and disability). This analysis was restricted to participants in the phenotype categories normal or successful aging. As reported in the Appendix 3, we ran several sensitivity analyses to examine potential competing risk bias and

the effect of sample selection and missing data on the associations and also tested the extent to which assessing the level of IL-6 at two time points -as compared to using a single measurement - provide a stronger predictor of future aging phenotypes(19, 20).

All analyses were conducted using the Statistical Analyses Software version 9 (Statistical Analyses Software Institute, Cary, North Carolina, USA).

RESULTS

Of the 3044 participants included in the present analyses, 23.7% (n=721) met the criteria for successful aging at the last follow-up, 10.6 % (n=321) had cardiovascular disease events (42 fatal and 279 non-fatal) and 4.8% (n=147) died from non-cardiovascular causes. The remaining 60.9 % (n=1855) formed the normal aging phenotype group. Characteristics of participants were compared across these 4 aging phenotypes and are described in Table 1. Compared to the normal aging group, participants in the successful aging group were more likely to have low interleukin-6 levels at baseline, while those who developed a cardiovascular disease event or died from non-cardiovascular causes were more likely to have high interleukin-6 levels.

Interleukin-6 levels at baseline and over the 5-year exposure period (measured 5-y before baseline and at baseline) were significantly associated with future aging phenotypes in sex and aged adjusted analyses (Table 2). After further adjustment for potential confounders (Figure 2), participants with high interleukin-6 at baseline had half the odds of achieving successful aging at 10-year follow-up (odds ratio ((OR))=0.45, 95 % confidence interval (CI): 0.35-0.59) and showed respectively, a 1.76- and 2.64-fold odds of developing cardiovascular disease (non-fatal/fatal, 95% CI: 1.21-2.55) or dying from non-cardiovascular causes (95% CI: 1.53-4.55) over the 10-year follow-up. Participants with a high interleukin-6 level on 0, 1, and 2 occasions over the 5-year exposure period had an OR of 1.00 (reference), 0.66 (95% CI: 0.53-0.82) and 0.53 (95% CI: 0.38-0.74) for successful aging ($p_{trend}<0.001$) (Figure 2). Figure 2 also shows that having high interleukin-6 levels twice over the 5-year exposure period was associated with an increased odds of developing cardiovascular disease (OR=1.64, 95 % CI: 1.15-2.33, $p_{trend}=0.002$) and dying from non-cardiovascular causes (OR=2.43, 95 % CI: 1.56-3.80, $p_{trend}<0.001$). When

interleukin-6 was replaced by C-reactive protein, similar significant associations with aging phenotypes were also observed (Appendix 4).

Further analyses examined which specific successful aging component characterized the inflammation-successful aging phenotype relationship. The results, presented in Table 3, show that high interleukin-6 levels at baseline were associated with lower odds of good cardiovascular, respiratory and musculoskeletal functioning, and good mental well-being as well as lower odds of remaining free of diabetes and disability. When inflammation was assessed over the 5-year exposure period trends were observed and having high interleukin-6 twice over the 5-year exposure was associated with reduced odds of absence of diabetes, good respiratory functioning and good musculoskeletal functioning components of successful aging

Sensitivity analyses

Repeating analyses using multinomial regression to examine potential competing risk bias led to findings similar to those in the main analysis, suggesting that competing risk bias was unlikely (Appendix 5). In further sensitive analyses the results did not materially change after excluding (1) obese participants (defined by a body mass index $\geq 30 kg/m^2$); (2) those on anti-inflammatory medications and (3) participants with acute inflammation (C-reactive protein >10 mg/L) (Appendix 6). The OR for inflammation and non-cardiovascular mortality among the 5353 participants with complete data on inflammation and cause of death and the 3044 participants included in the main analyses were also largely similar. Furthermore, in analysis using multiple imputation to deal with missing data on inflammation and covariates, comparable associations to those reported in the main analyses were obtained. Finally, the net reclassification improvement statistics showed that information from two measurements of inflammation

improved the risk prediction for successful aging by 23.6%, for cardiovascular events by 15.4% and for non-cardiovascular mortality by 22.1% (Appendix 7).

INTERPRETATION

Main findings

We showed that chronic exposure to high levels of interleukin-6 halved the odds of successful aging ten years later and wsa associated with increased odds of future cardiovascular disease and non-cardiovascular mortality in a dose-response fashion. These associations were found to be independent of socio-economic factors, health behaviours (smoking, physical activity), and conditions such as obesity as well as the use of anti-inflammatory drugs and acute inflammation.

Comparison with other studies and explanation

Most previous studies have investigated the impact of inflammation on cardiovascular and mortality outcomes based on inflammation measured at only one point in time(4). A recent collaborative meta-analysis suggested that prolonged increases in interleukin-6 levels were associated with risk of coronary heart disease(10). In that report the long-term average impact of interleukin-6 was estimated from a single measure of the inflammatory marker using a "regression dilution ratio" method. Our study using repeated measures of interleukin-6 confirmed this finding. Three studies have reported on an association between change in interleukin-6 levels and age-related health outcomes. Two studies, carried out on 860(21) and 736(22) older adults, showed that short-term (3-year) and long-term (9-year) increases in interleukin-6 were associated with an increased 3-year risk of overall mortality. A further study based on the Cardiovascular Health All Stars Study (n=840)(23) showed that the 9-year increase in interleukin-6 was also associated with an increased risk of cardiovascular disease. Our results on the associations between inflammation, cardiovascular events and mortality are concordant

with those reported in the literature. However, our results also show that measuring chronic inflammation using two repeated measures may be a better predictor of future cardiovascular disease and non-cardiovascular mortality, than measuring inflammation only once.

We found that chronic inflammation characterized by high levels of interleukin-6 (>2 pg/mL) twice over the 5-year exposure period nearly halved the odds of successful aging after 10–years of follow-up compared to maintaining low levels of interleukin-6 (<1pg/mL twice over the exposure period). Our study indicates that high interleukin-6 levels at baseline were inversely associated with most of the components that characterise successful aging; good cardiovascular, respiratory, and musculoskeletal functioning, good mental well-being and the absence of diabetes and disability. Exceptions were components related to good cognitive function and cancer. Regarding cognition, a more robust association with inflammation was expected according to available epidemiological literature which provides support for an inflammation-cognitive decline relationship(5). In contrast, the non-significant inflammation-cancer association reported in our study is in accordance with the results of the British Women's Heart and Health Study(24) which showed that in a cohort of 4,286 women aged 60 to 79 years, elevated interleukin-6 concentrations were similarly associated with an increased risk of death in elderly women with and without cancer, suggesting that these inflammatory markers were likely to be indicators of non-cancer comorbidities rather than related to the malignancy itself(24). Furthermore, our results also indicate that low levels of inflammation may facilitate successful aging by lower likelihood of impaired respiratory and musculoskeletal functioning and by preventing diabetes onset.

Limitations

This study has some important limitations. First, as our study was comprised exclusively of middle-aged London-based civil servants at baseline, findings may have limited generalisability to the total British population(15). Second, analyses were carried out on participants with complete data on aging outcomes and inflammatory markers constituting only 53 % of the 5706 participants at baseline (Figure 1). However, comparable results observed in the supplementary analyses - one including all participants for whom mortality records were available and the other using multiple imputation methods to deal with missing data - suggest that a major bias due to sample selection is an unlikely explanation for our results. Third, we used two measurements of interleukin-6 over the exposure period (5-y before baseline and at baseline) to assess long-term inflammation. Although we showed that the predictive ability of the aging phenotype is significantly better when inflammation is assessed with measures repeated at two time points rather than one, having a high interleukin-6 level at the beginning and end of a 5-year exposure period should not necessarily be regarded as an indicator of chronic inflammation as repeated short-term inflammatory responses are also possible. Finally common to other observational studies and despite adjustment for socio-demographic and health behaviour factors, we cannot exclude the possibility that the inflammatory-aging phenotype relationships observed may be partly explained by residual confounding.

Conclusion and implications for practice and future research

Despite these limitations, by analysing aging phenotypes based on validated clinic-based measures and medical records, in a large sample with a long follow-up, we were able to show that chronic inflammation, as ascertained by repeat measurements, appears to be related to a range of unhealthy aging phenotypes and may facilitate successful aging mostly by lower

likelihood of impaired respiratory and musculoskeletal functioning and by preventing diabetes onset. If confirmed, these results shed new light on the importance of assessing long-term chronic inflammation in geriatric clinical practice to target individuals at risk to develop unhealthy aging but also to promote ideal health by managing long-term chronic inflammation.

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FIGURE LEGENDS

Figure 1: Flow chart mapping the selection of the 3044 Whitehall II participants included in the present analyses.

* To allow follow-up beyond age 60 for surviving participants

Figure 2: Multivariable-adjusted* association between interleukin-6 levels at baseline and over the 5-year exposure period and subsequent aging phenotypes over the 10-year follow-up.

A Interleukin-6 levels at baseline**
Caption: OR 95 % CI

B No. of times interleukin-6 was high over the 5-y exposure period †
Caption: OR 95 % CI

Footnotes

*Results from logistic regression adjusted for sex, age, socio-economic status, smoking status, physical activity, acute inflammation and use of anti-inflammatory drugs
Analyses assessed the associations of inflammation with:
(1) Successful aging (non-cases: normal aging phenotype, cardiovascular disease events and non-cardiovascular death combined), total n=3044;
(2) Cardiovascular disease events (non-cases: successful and normal aging phenotypes), total n=2897;
(3) Non-cardiovascular death (non-cases: successful and normal aging phenotypes), total n=2723.
**Distribution of Interleukin-6 at baseline was divided into three categories: low (≤1.0pg/mL), intermediate (1.1-2.0pg/mL) and high (>2.0pg/mL).
† Interleukin-6 was measured twice (5 years before baseline and at baseline). A value of "2" indicates both measurements were high, a value of "1" that either measurement was high and a value of "0" indicates that none of these measurements was high.

Table 1: Characteristics of participants at baseline (1997-99) according to aging phenotype at the 10-year follow-up

	Study group by outcome; no. (%) or mean ± SD at baseline					
	|All	**Successful Aging**	**Normal Aging**	**CVD Events**	**Non CVD Death**	**p-value***
		721 (23.7)	1855 (60.9)	321 (10.6)	147 (4.8)	
Age, y, mean (SD)		56.1 ± 5.0	56.9 ± 5.1	59.6 ±5.2	59.5 ±5.3	0.001
Sex, women	818 (28.9)	180 (25.0)	533 (28.7)	59 (18.4)	46 (31.3)	0.006
Socio-economic status						
Low grade	371 (12.2)	41 (5.7)	266 (14.3)	38 (11.8)	26 (17.7)	<0.001
Intermediate grade	1275 (41.9)	269 (37.3)	807 (43.3)	140 (43.6)	59 (40.1)	
High grade	1398 (45.9)	411 (57.0)	782 (42.2)	143 (44.6)	62 (42.2)	
Smoking Status						
Never	1580 (51.9)	409 (56.7)	957 (51.6)	152 (47.3)	62 (42.2)	<0.001
Former	1213 (39.9)	276 (38.3)	739 (39.8)	139 (43.3)	59 (40.1)	
Current	251 (8.2)	36 (5.0)	159 (8.6)	30 (9.3)	26 (17.7)	
Physical activity						
Inactive	852 (28.0)	167 (23.2)	547 (29.5)	88 (27.4)	50 (34.0)	0.007
Moderately active	519 (17.0)	122 (16.9)	322 (17.4)	47 (14.6)	28 (19.1)	
Active	1673 (55.0)	432 (59.9)	986 (53.1)	186 (57.9)	69 (46.9)	
Anti-inflammatory drugs	295 (9.7)	37 (5.1)	193 (10.4)	41 (12.8)	24 (16.3)	<0.001
Acute inflammation	86 (2.8)	9 (1.2)	60 (3.2)	13 (4.0)	4 (2.7)	0.02
Obesity**	361 (11.9)	46 (6.4)	249 (13.4)	40 (12.4)	26 (17.7)	<0.001

Use of lipid lowering drugs**	75 (2.5)	15 (2.1)	46 (2.5)	11 (3.4)	3 (2.1)	0.4
Interleukin-6 levels at baseline						
Low (≤1.0pg/mL)	756 (24.8)	257 (35.6)	431 (23.2)	49 (15.3)	19 (12.9)	<0.001
Inter (1.1-2.0pg/mL)	1456 (47.8)	344 (47.7)	896 (48.3)	158 (59.2)	58 (39.5)	
High (>2.0pg/mL)	832 (27.4)	120 (16.6)	528 (28.5)	114 (35.5)	70 (47.6)	
No. of times interleukin-6 was high over the 5-y exposure period†						
0	1867 (61.3)	526 (72.9)	1110 (59.8)	164 (51.1)	67 (45.6)	<0.001
1	791 (26.0)	145 (20.1)	507 (27.3)	98 (30.5)	41 (27.9)	
2	386 (12.7)	50 (6.9)	238 (12.8)	59 (18.4)	39 (26.5)	

* To compare the characteristics among the 4 outcome group, the Chi-square test were used for categorical variables and the Student t-test were used for age variable.
**373 participants had missing value for body mass index and 10 participants had missing value for use of lipids lowering drugs.
† Interleukin-6 was measured twice (5 years before baseline and at baseline). A value of "2" indicates both measurements were high, a value of "1" that either measurement was high and a value of "0" indicates that none of these measurements was high.

Table 2: Sex and age adjusted associations between interleukin-6 levels assessed at baseline and over the 5-year exposure period and aging phenotypes at 10-year follow-up*

	Successful aging, n=721			Fatal or Non Fatal cardiovascular disease event, n=321				Non-cardiovascular death, n=147			
	N cases	OR	95 %CI	*N cases*	OR	95 %CI	P	*N cases*	OR	95 %CI	p
Interleukin-6 levels at baseline											
Low (≤1.0pg/mL)	*257*	1	Ref	*49*	1	Ref		*19*	1	Ref	
Intermediate (1.1-2.0pg/mL)	*344*	0.64	0.53;0.78	*158*	1.47	1.04;2.06		*58*	1.39	0.81;2.36	
High (>2.0pg/mL)	*120*	0.36	0.28;0.46	*114*	1.97	1.37;2.82		*70*	3.06	1.80;5.18	
No. of times interleukin-6 was high over the 5-y exposure period**											
0	*526*	1	Ref	*164*	1	Ref		*67*	1	Ref	
1	*145*	0.58	0.47;0.72	*98*	1.47	1.12;1.93		*41*	1.48	0.99;2.21	
2	*50*	0.42	0.31;0.58	*59*	1.54	1.30;2.35		*39*	2.75	1.80;4.20	

*Logistic regression analyses adjusted for age and sex assessed associations of inflammation with:
(1) successful aging (non-cases: normal aging phenotype, cardiovascular events and non-cardiovascular death combined), total n=3044

(2) fatal or non-fatal cardiovascular disease events (non-cases: successful and normal aging phenotypes combined), total n=2897. The 147 participants with a non-cardiovascular death were excluded from this analysis.
(3) non-cardiovascular disease deaths (non-cases successful and normal aging phenotypes combined), total n=2723. The 321 participants with cardiovascular disease events were excluded from this analysis.

**Interleukin-6 was measured twice (5 years before baseline and at baseline). A value of “2” indicates both measurements were high, a value of “1” that either measurement was high and a value of “0” indicates that none of these measurements was high.

Table 3: Associations between interleukin-6 levels assessed at baseline and over the 5-year exposure period and components of successful aging at 10-year follow-up in 2576 participants*.

	Components of successful aging; adjusted OR (95% CI)**															
	Good cardiovascular functioning		Absence of diabetes		Good respiratory functioning		Good musculoskeletal functioning		Good cognitive functioning		Good mental well being		Absence of cancer		Absence of disability	
Interleukin-6 levels at baseline†																
Low	1.00	Ref	1.00	Ref	1.00	Ref	1.00	Ref	1.00	Ref	1.00	Ref	1.00	Ref	1.00	Ref
Int.	0.93	0.73;1.19	0.52	0.37;0.75	0.80	0.60;1.07	0.77	0.59;1.00	1.03	0.79;1.36	0.71	0.49;1.04	0.62	0.44;0.89	0.71	0.53;0.96
High	0.74	0.56;0.98	0.35	0.24;0.51	0.53	0.39;0.72	0.62	0.45;0.83	0.93	0.68;1.28	0.56	0.37;0.86	0.79	0.52;1.20	0.62	0.44;0.86
P trend	0.04		<0.001		<0.001		0.001		0.7		0.01		0.3		<0.001	
No. of times interleukin-6 was high over the 5-y exposure period‡																
0	1.00	Ref	1.00	Ref	1.00	Ref	1.00	Ref	1.00	Ref	1.00	Ref	1.00	Ref	1.00	Ref
1	0.72	0.58;0.90	0.66	0.51;0.87	0.71	0.55;0.92	0.86	0.68;1.09	0.79	0.62;1.01	0.80	0.58;1.11	1.15	0.83;1.59	0.89	0.69;1.15
2	0.76	0.55;1.06	0.44	0.31;0.62	0.41	0.30;0.58	0.67	0.49;0.93	0.79	0.56;1.12	0.71	0.46;1.09	0.82	0.54;1.23	0.80	0.57;1.13
P trend	0.01		<0.001		<0.001		0.02		0.06		0.07		0.7		<0.001	

*Participants who developed a fatal and non-fatal cardiovascular disease event and those who died from a non-cardiovascular cause (n=468) were excluded from these analyses.

**Odds ratios from logistic regression are adjusted for sex, age, socio-economic status, smoking status, physical activity, acute inflammation and use of anti-inflammatory drugs.
†Distribution of Interleukin-6 at baseline was divided into three categories: low (≤1.0pg/mL), intermediate (1.1-2.0pg/mL) and high (>2.0pg/mL).
‡ Interleukin-6 was measured twice (5 years before baseline and at baseline). A value of “2” indicates both measurements were high, a value of “1” that either measurement was high and a value of “0” indicates that none of these measurements was high