Inflammatory biomarkers in midlife as predictors of all-cause, cardiovascular, and cancer mortality:

Results from the Whitehall II cohort Study.

Archana Singh-Manoux PhD[1*], Martin J Shipley MSc[2], Joshua A. Bell PhD[2], Marianne Canonico PhD[1], Alexis Elbaz MD, PhD[1], Mika Kivimäki PhD[2]

*[1] INSERM, U1018, Centre for Research in Epidemiology and Population Health, Université Paris-Saclay
Hôpital Paul Brousse, Bât 15/16, 16 Avenue Paul Vaillant Couturier
94807 VILLEJUIF CEDEX, France
Telephone: +33 (0)1 77 74 74 10 Fax: +33 (0)1 77 74 74 03
Email: Archana.Singh-Manoux@inserm.fr

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

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Author Statements

ASM, MJS, and MK developed the hypothesis and study design. MJS performed statistical analysis. ASM wrote the first and successive drafts of the manuscript. All authors contributed to study concept and design, analysis and interpretation of data, and drafting or critical revision of the manuscript for important intellectual content.

Conflicts of Interest.

The authors have no conflicts of interest to declare.

Abstract

Background: Alpha-1-acid glycoprotein (AGP), an inflammatory marker, was found to have the strongest association with 5-year mortality in a recent study that examined 106 biomarkers. Our objective was to examine whether it is a better marker of mortality risk than more commonly used inflammatory biomarkers C-reactive protein (CRP) and interleukin-6 (IL-6).

Methods: We analyzed data on 6545 men and women aged 45-69 (mean 55.7) years from the Whitehall II cohort study. Biomarkers (AGP, CRP, and IL-6) were assayed from fasting serum collected in 1997-1999. Mortality follow-up was until June, 2015. Cox regression was used to model associations of inflammatory markers with all-cause, cancer, and cardiovascular mortality.

Results: Over the mean follow-up of 16.7 years, 736 deaths occurred, of which 181 were from cardiovascular disease and 347 from cancer. In models adjusted for age, sex, BMI, health behaviors and chronic disease (prevalent coronary heart disease, type 2 diabetes, any cancer type and chronic obstructive pulmonary disease), AGP did not predict mortality beyond the first 5 years of follow-up, over this period IL-6 and CRP had stronger associations with mortality. When considering all covariates and biomarkers simultaneously, AGP no longer predicted all-cause mortality (HR=0.99, 95% CI 0.90-1.08). Only IL-6 predicted all-cause (HR=1.22, 95% CI: 1.12-1.33) and cancer mortality (HR=1.13, 95% CI: 1.00-1.29) over the entire follow-up, while CRP predicted only cardiovascular mortality (HR=1.30, 95% CI: 1.06-1.61).

Interpretation: Our analyses suggest alpha-1-acid glycoprotein is not a better marker of short- or long-term mortality risk than commonly used interleukin-6 and C-reactive protein.

Inflammatory biomarkers are useful indicators of infection in care settings(1) and have great value for monitoring chronic disease activity(2) and overall health status in the wider population.(3) Considerable research shows inflammatory markers to predict mortality in adults with chronic conditions such as type 2 diabetes(4) and cardiovascular disease,(5) while interleukin-6 (IL-6) and C-reactive protein (CRP) have also been shown to predict mortality and cardiovascular outcomes in general population settings.(6) In older adults, IL-6 appears to have a stronger association than CRP with all-cause mortality.(3) For cardiovascular outcomes, Mendelian randomization studies suggest causal effects for IL-6 but not CRP.(7, 8)

A recent metabolomics study examined 106 biomarkers and found alpha-1-acid glycoprotein (AGP), an acute phase protein, to be the strongest predictor of five-year mortality.(9) The importance of this finding has not been established for mortality follow-up beyond five years. It is also unknown how well AGP compares with other sensitive, dynamic, and commonly measured markers of systemic inflammation, such as CRP and IL-6, as a predictor of mortality. To address this question, we compared associations of these three inflammatory markers with short- and long-term risk of all-cause, cardiovascular- and cancer-related mortality in a large cohort study. We also examined absolute differences in life expectancy at age 50 in those with high versus low inflammation based on each marker.

Methods

The Whitehall II study is a cohort study of men and women originally employed by the British civil service in London-based offices. (10) A total of 10,308 persons (6895 men and 3413 women, aged 35-55) were recruited to the study over the years 1985 to 1988, with a response rate of 73%.(9) Since the baseline medical examination, follow-up examinations have taken place approximately every 5 years. Ethical approval was obtained from the University College London Medical School committee on the ethics of human research (reference number 85/0938); all participants provided written informed consent.

Assessment of IL-6, CRP, and AGP

Samples of fasting serum for inflammatory markers were collected in 1997-1999. CRP was measured with a high-sensitivity immunonephelometric assay in a BN ProSpec nephelometer (Dade Behring, Milton Keynes, UK); IL-6 with a high-sensitivity ELISA (R&D Systems, Oxford, UK); and AGP using nuclear magnetic resonance spectroscopy as part of more complete biomarker profiling.(11)

Mortality

Mortality data until June 2015 were drawn from the British national mortality register (National Health Services Central Registry). The tracing exercise was carried out using the National Health Service identification number of each participant. Besides all-cause mortality, the 9th and 10th revisions of the

International Classification of Disease were used to examine deaths due to cardiovascular-- (ICD-9 codes 390–459 and ICD-10 codes I00-I99) and cancer- (ICD-9 codes 140–208 and ICD-10 codes C00-C97) related causes.

Covariates

Covariates used in the analyses were drawn from the 1997-1999 assessment, concurrent to the measurement of inflammatory markers. Demographic covariates included age, sex, and employment grade – a 3-level marker of socioeconomic status (SES) in the Whitehall II study.(10) Health behavior covariates included smoking status (categorized as current, ex-, and never smoker), alcohol consumption (assessed via questions on the number of alcoholic drinks consumed in the last week and converted to units of alcohol), physical activity level (defined as 'active' for ≥2.5 hours/week of moderate physical activity or ≥1 hour/week of vigorous physical activity, 'inactive' for <1 hour/week of moderate and vigorous activity and 'intermediate' for all others), and dietary behavior (assessed using a question on frequency of fruit and vegetable consumption in a typical week). Body mass-index (BMI) was measured as weight, kilograms/height in metres^2 and used as a continuous variable. Chronic disease burden was assessed by prevalence of coronary heart disease (CHD) and stroke (CHD included definite non-fatal myocardial infarction and definite angina cases based on questionnaires, study electrocardiograms, and use of cardiac enzymes; stroke was self-reported; as far as possible, all identified cases were corroborated via linkage to the UK Hospital Episode Statistics database which contains data on all in- and out-patient treatment); chronic obstructive pulmonary disease (MRC respiratory questionnaire(12) self-reported), cancer (assessed using the National Health Service cancer registry); and type 2 diabetes mellitus (determined by fasting glucose ≥ 7.0 mmol/l and/or 2-h postload glucose ≥ 11.1 mmol/l, self-report of doctor diagnosis or diabetes medication).(13)

Statistical analyses

We examined participants' baseline characteristics as a function of vital status at the end of follow-up and summarized the associations using standardized differences. Inflammatory markers were log-transformed owing to skewed distributions and standardized to z-scores to allow comparison of effect sizes. 590 individuals (9.0%) had missing values for one or more variables which were imputed using multiple imputation to generate ten datasets which were analyzed separately with the results being combined using Rubin's rules.(14)

For survival analyses, participants were followed until death or until the date of censoring for those who were alive (date of emigration or June 30th, 2015), whichever occurred first. Cox proportional hazards models with years of follow-up as the underlying time variable were used to examine hazard ratios (HR)

and their 95% confidence intervals (CI) for associations with mortality. Schoenfeld's residuals suggested violation of the proportionality of hazards assumption, justifying our use of three follow-up periods for mortality outcomes: 0-5y, 5-10y, ≥10y. There were no sex differences in associations between inflammatory markers and mortality, p for interaction ranged from 0.16 to 0.42, leading us to combine men and women in analyses. The first model adjusted for age and sex (Model 1). The second model additionally adjusted for SES, BMI, health behaviors, and prevalent chronic disease (Model 2). The final model included all three inflammatory markers along with covariates (Model 3).

In order to take into account possible threshold effects, analyses with mortality were repeated using tertiles for each inflammatory marker (comparing highest versus lowest). Using these same tertiles, we estimated remaining life expectancy at age 50 for men and women with high versus low inflammation by multiplying the England and Wales age-specific mortality rates for 2011-2013 with the hazard ratios for the inflammatory marker-mortality association in the two groups compared with the total sample. These analyses were undertaken separately in men and women due to known sex differences in life expectancy.

All analyses were undertaken using SAS 9.2 (SAS Institute, NC, USA). Statistical tests were 2-sided with a P-value<0.05 considered statistically significant.

Results

A total of 6551 participants underwent clinical assessment in 1997-99, six of whom were not linked to mortality records and were excluded from all analyses. Mean (range) age of participants at the 1997-99 clinical assessment was 55.7 (45-69) years. A total of 736 deaths occurred over a mean follow-up of 16.7 years, 14.5% in the first five years, and 61.7% more than ten years after assessment of biomarkers. Those who died were older (59.6 vs 55.2 years) but did not differ by sex (Table 1). They had an adverse socioeconomic and disease profile, including higher levels of all three inflammatory markers (Table 1). AGP was correlated with IL-6 ($r=0.29$, $p<0.001$) and CRP ($r=0.33$, $p<0.001$); IL-6 and CRP were also correlated with each other ($r=0.46$, $p<0.001$).

In models adjusted for age and sex (Model 1), all three inflammatory biomarkers were associated with all-cause mortality (Table 2). In addition, all associations weakened over time and were stronger for deaths occurring in the first five years than for those occurring ≥10 years. In models adjusted for all covariates (Model 2), AGP did not predict mortality beyond the first five years of follow-up. In analyses considering all three biomarkers simultaneously (Model 3), only IL-6 predicted short- and long-term mortality from all causes, the overall hazard ratio for the entire follow-up being 1.22 (95% CI: 1.12, 1.33). Table 3 shows the

unadjusted and mutually adjusted associations of all covariates with all-cause mortality, the mutually adjusted model corresponded to Model 3 in Table 2 for inflammatory markers.

A total of 181 (24.6%) deaths were attributed to cardiovascular disease, for which all three markers were significant predictors in models adjusted for age and sex (Model 1, Table 2), although the association with AGP was driven primarily by deaths in the first five years of follow-up (HR=1.43 for deaths in the first five years compared with 1.09 for deaths after 10 years, Table 2). In analyses adjusted for covariates and mutually adjusted for the three inflammatory markers (Model 3), only CRP remained associated with cardiovascular deaths (HR=1.30, 95% CI: 1.06, 1.61). This pattern was also observed in analyses stratified by duration of follow-up.

Of the deaths, 47.1% were due to cancer. All three markers were associated with cancer deaths in models adjusted for age and sex (Model 1), although not beyond the first five years for AGP (Table 2). In the fully adjusted model including all three inflammatory markers (Model 3), only IL-6 was associated with cancer mortality (HR=1.13, 95% CI: 1.00, 1.29).

In order to take into account possible threshold effects, mortality risk was compared in the top versus the bottom tertile of each inflammatory marker. As shown in Table 4, no association was observed between AGP and mortality in models adjusted for all covariates (Models 2 and 3). On the other hand, IL-6 was associated with all-cause mortality in all models, irrespective of the covariates included.

Figure 1 shows remaining life expectancy at age 50 in participants with high compared with low inflammation, defined using the highest compared with the lowest tertile. For AGP, there was a non-statistically significant difference in remaining life expectancy in men and women of 1.0 year (95% CI: 0.0, 2.0), p=0.054 and 0.06 in men and women. The difference in remaining life expectancy at age 50 (Figure 1) was significantly lower in those in the highest tertile of IL-6 (3.8 years; 95% CI: 2.9, 4.7) and CRP (2.7 years; 95% CI: 1.6, 3.7).

Interpretation

Main findings

This analysis examined the association of three inflammatory markers with short- and long-term mortality in a large sample of middle-aged adults, followed for 17 years. Analyses were motivated by a recent molecular profiling study that assessed the association of 106 metabolites with mortality and found AGP to

have the strongest association with all-cause mortality.(9) As AGP is an acute phase protein which is not widely measured,(15) we compared its predictive ability with more commonly measured inflammatory markers for mortality. Our results show that AGP was indeed associated with all-cause and cancer mortality in the short term; however it was not associated with mortality in the longer term. Furthermore, when examining all three inflammatory markers in mutually adjusted models, AGP was not associated with mortality even in the short term. Thus, our analyses provide no evidence that AGP would be a better marker of mortality risk than more commonly used inflammatory indicators, IL-6 or CRP.

In analysis adjusted for all covariates where each marker was examined separately, both CRP and IL-6 were associated with all-cause and cancer mortality over the 17-year follow-up, with CRP also associated with cardiovascular mortality. However, when all inflammatory markers were considered together, only IL-6 was associated with all-cause and cancer mortality and CRP with cardiovascular mortality. Identification of inexpensive prognostic markers for ill-health in vulnerable populations, the elderly, or those with chronic conditions is important for management of care, and a better understanding of the role of inflammatory markers in general population settings may help clarify their utility for guiding screening and prevention. Interactions between environmental, stochastic, genetic and epigenetic factors shape mortality risk. There is considerable interest in identifying biological markers of such risk, in particular those that can be measured non-invasively. Plasma biomarkers are ideal for this purpose although observed associations do not necessarily reflect causality. In our data, CRP outweighed IL-6 as a predictor of cardiovascular mortality although evidence from Mendelian randomization studies suggests a causal role for IL-6(16) but not CRP(17) in cardiovascular disease. Reanalysis of our data using tertiles of inflammatory markers rather than standardized z-scores suggested a robust association only between IL-6 and all-cause mortality.

Plasma concentrations of acute phase proteins fluctuate in response to inflammation. IL-6 is a major proinflammatory cytokine, produced in a variety of tissues, and CRP and AGP are downstream products of the acute phase response, derived via cytokine dependent hepatic biosynthesis and secretion into the systemic circulation.(18) Thus, IL-6 might be a more appropriate marker of long-term health status. There is considerable interest in the importance of inflammatory pathways for ageing outcomes.(19-21) It is possible that chronic low-grade inflammation plays a role in neurodegenerative diseases. This is supported by genome wide association studies showing a number of genetic variants that influence inflammatory pathways to be associated with development of Alzheimer's disease.(22, 23)

Comparison to previous studies

In HIV-infected pregnant women, higher AGP is associated with increased risk of maternal death, postnatal transmission, and infant infection or death, possibly due to the impact of AGP on modulating immunity and

binding or carrying drugs.(1) There is little research on AGP and health status in general population samples, the exceptions being the study on metabolites and 5-year mortality where AGP was one of the 106 biomarkers (no other inflammatory markers were examined in that study)(9) and those on mortality risk in healthy and hospitalized elderly.(24, 25) However, previous studies comparing AGP with other inflammatory markers for their associations with short- and long-term mortality are lacking. Our results show a robust association of AGP with 5-year risk of all-cause, cardiovascular and cancer mortality in analyses adjusted for age and sex, with the magnitude of effect being similar to previous reports.(9) However, by considering a longer follow-up and other inflammatory markers simultaneously, we showed that IL-6 and CRP are more important predictors of mortality, and that AGP does not provide additional information regarding mortality.

Strengths and limitations

A key strength of this study, compared to previous studies, is the extended follow-up for mortality in a large cohort of men and women. We were also able to examine cancer and cardiovascular mortality. Limitations of our study include the lack of ethnic diversity in the population. However, generalizability is unlikely to be compromised as the study was designed to include a wide socioeconomic spectrum, with over a 10-fold salary difference across the socioeconomic hierarchy. We have previously shown associations of common risk factors with CVD incidence in our study to be comparable to studies based on general population samples.(26) A further limitation is the use of only three serum markers of systemic inflammation. CRP and IL-6 are widely used in clinical practice and CRP is particularly inexpensive to measure, although it remains unclear whether there exist more relevant markers of inflammation.

Conclusions

We found no evidence that AGP is a stronger prognostic marker of mortality than the widely used inflammatory markers, IL-6 and CRP. As with previous findings, AGP was associated with 5 year mortality but even with this length of follow-up it did not do better than IL-6 in predicting mortality. Our analysis of all-cause, cancer and cardiovascular mortality suggests that for all these outcomes IL-6 may be a better prognostic marker, both in the short and the long-term.

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Table 1: Sample characteristics at start of follow-up (1997-1999)

	Status at end of follow-up (06_ 2015)		Standardized differe avence[a]
	Alive (N=5809)	Deceased (N=736)	
Characteristics in 1997-1999			
Age, Mean (SD)	55.2 (5.9)	59.6 (5.8)	0.75
Men, %	70.9	71.1	0.00
Low SES, %	13.2	17.9	0.13
Current Smoker, %	9.0	16.1	0.22
Units of alcohol consumption per week, Mean (SD)	13.6 (15.0)	14.8 (18.9)	0.07
Inactive,[b] %	8.1	8.5	0.02
Daily consumption of fruits & vegetables, %	73.8	69.7	-0.09
BMI, Mean(SD)	26.1 (3.9)	26.7 (4.6)	0.16
History of CVD (CHD or Stroke), %	5.4	13.2	0.27
History of diabetes, %	5.6	12.5	0.24
History of cancer, %	2.3	5.0	0.14
History of COPD, %	7.5	11.9	0.15
Inflammatory markers (standardized values),[c] Mean (SD)			
Alpha-1-acid glycoprotein (AGP)	-0.02 (0.99)	0.20 (1.05)	0.22
Interleukin-6 (IL-6)	-0.05 (0.98)	0.42 (1.04)	0.47
C-reactive protein (CRP)	-0.03 (0.99)	0.27 (1.03)	0.30

SD: Standard Deviation

[a] Standardized differences greater than 0.1 are considered meaningful

[b] Inactive' for <1 hour/week of moderate and vigorous activity

[c] The median (inter quartile range) of each biomarker prior to standardization in the alive and deceased group are as follows:

AGP: 1.42 (1.29, 1.57) and 1.47 (1.33, 1.62)

IL-6: 1.38 (0.98, 2.04) and 1.84 (1.26, 2.82)

CRP: 0.99 (0.50, 2.06) and 1.36 (0.64, 6.87)

Table 2: Associations of inflammatory biomarkers with all-cause, CVD and cancer mortality[a]

		Model 1 Adjusted for age and sex			**Model 2** Adjusted for age, sex, employment grade, BMI, health behaviors, and chronic disease			**Model 3** Adjusted for all covariates and other inflammatory markers		
Follow -up	**Deaths/ N**	**AGP** HR (95% CI)	**IL-6** HR (95% CI)	**CRP** HR (95% CI)	**AGP** HR (95% CI)	**IL-6** HR (95% CI)	**CRP** HR (95% CI)	**AGP** HR (95% CI)	**IL-6** HR (95% CI)	**CRP** HR (95% CI)
All-cause mortality										
ALL	**736/6545**	**1.18 (1.09,1.27)**	**1.35 (1.26,1.44)**	**1.27 (1.17,1.37)**	**1.06 (0.97,1.15)**	**1.25 (1.16,1.35)**	**1.17 (1.07,1.27)**	**0.99 (0.90,1.08)**	**1.22 (1.12,1.33)**	**1.07 (0.97,1.18)**
0-5 y	107/6545	1.43 (1.19,1.72)	1.75 (1.49,2.06)	1.65 (1.36,2.00)	1.30 (1.06,1.59)	1.61 (1.36,1.92)	1.54 (1.25,1.90)	1.09 (0.87,1.37)	1.47 (1.20,1.81)	1.18 (0.93,1.51)
5-10 y	175/6438	1.24 (1.08,1.44)	1.36 (1.18,1.56)	1.28 (1.10,1.50)	1.15 (0.98,1.34)	1.28 (1.10,1.49)	1.21 (1.02,1.43)	1.06 (0.89,1.27)	1.23 (1.04,1.46)	1.06 (0.87,1.30)
≥10 y	454/6263	1.09 (0.99,1.20)	1.25 (1.14,1.37)	1.18 (1.08,1.31)	0.98 (0.88,1.09)	1.15 (1.04,1.27)	1.07 (0.96,1.20)	0.93 (0.83,1.05)	1.15(1.03,1.28)	1.04 (0.91,1.18)
Cardiovascular mortality[b]										
ALL	**181/6541**	**1.27 (1.10,1.46)**	**1.37 (1.19,1.57)**	**1.51 (1.30,1.76)**	**1.06 (0.90,1.24)**	**1.19 (1.02,1.39)**	**1.32 (1.12,1.57)**	**0.94 (0.79,1.13)**	**1.08 (0.90,1.29)**	**1.30 (1.06,1.61)**
0-5 y	31/6543	1.44 (1.02,2.05)	1.57 (1.14,2.16)	1.56 (1.08,2.24)	1.28 (0.87,1.87)	1.34 (0.96,1.88)	1.49 (1.00,2.22)	1.11 (0.72,1.71)	1.17 (0.78,1.76)	1.28 (0.78,2.11)
5-10 y	42/6436	1.32 (0.98,1.77)	1.51 (1.15,1.99)	1.63 (1.19,2.21)	1.06 (0.76,1.48)	1.36 (1.01,1.85)	1.39 (0.99,1.97)	0.92 (0.64,1.31)	1.25 (0.87,1.79)	1.28 (0.84,1.95)
≥10 y	108/6263	1.20 (1.00,1.45)	1.26 (1.04,1.51)	1.45 (1.19,1.77)	0.99 (0.81,1.22)	1.07 (0.86,1.33)	1.25 (1.00,1.57)	0.91 (0.72,1.14)	0.97 (0.76,1.24)	1.32 (1.00,1.75)
Cancer mortality[b]										
ALL	**347/6541**	**1.09 (0.98,1.22)**	**1.25 (1.13,1.39)**	**1.23 (1.11,1.38)**	**0.99 (0.88,1.12)**	**1.17 (1.04,1.30)**	**1.15 (1.02,1.30)**	**0.92 (0.81,1.06)**	**1.13(1.00,1.29)**	**1.12 (0.97,1.29)**
0-5 y	54/6543	1.50 (1.17,1.91)	1.66 (1.32,2.09)	1.78 (1.37,2.33)	1.35 (1.02,1.78)	1.52 (1.18,1.97)	1.64 (1.23,2.19)	1.13 (0.83,1.53)	1.30 (0.96,1.76)	1.34 (0.95,1.90)
5-10 y	87/6436	1.11 (0.90,1.38)	1.21 (0.98,1.48)	1.17 (0.94,1.46)	1.01 (0.79,1.28)	1.11 (0.88,1.40)	1.07 (0.84,1.38)	0.97 (0.75,1.27)	1.09 (0.85,1.41)	1.04 (0.78,1.39)
≥10 y	206/6263	0.99 (0.85,1.15)	1.17 (1.02,1.34)	1.14 (0.99,1.32)	0.90 (0.76,1.07)	1.10 (0.95,1.28)	1.08 (0.92,1.27)	0.85 (0.71,1.02)	1.10 (0.93,1.30)	1.10 (0.91,1.34)

AGP: alpha-1-acid glycoprotein, IL-6: Interleukin-6, CRP: C-reactive protein (CRP).
[a]Hazard ratios (HR) are per 1-standard deviation increase in the inflammatory biomarker.
[b] Four participants with unknown cause of death have been excluded from these analyses

Table 3: Hazard ratios for inflammatory biomarkers and baseline covariates for all-cause mortality[a]

	Unadjusted	Mutually adjusted
	HR (95% CI)	HR (95% CI)
Age, (per year)	1.12 (1.11, 1.14)	1.11 (1.10, 1.13)
Sex, (Women vs Men)	0.98 (0.84, 1.15)	0.87 (0.72, 1.04)
SES		
Intermediate vs High	1.04 (0.89, 1.22)	0.98 (0.83, 1.16)
Low vs High	1.41 (1.15, 1.73)	0.96 (0.75, 1.24)
Smoking		
Ex-smokers vs Never smokers	1.20 (1.03, 1.41)	1.07 (0.93, 1.30)
Current smokers vs Never smokers	2.08 (1.68, 2.56)	1.86 (1.49, 2.38)
Alcohol consumption		
None vs Moderate	1.29 (1.06, 1.57)	1.12 (0.91, 1.38)
Heavy vs Moderate	1.04 (0.88, 1.22)	1.10 (0.93, 1.30)
Physical activity		
Inactive vs Active	1.08 (0.84, 1.41)	0.94 (0.72, 1.24)
Moderately active vs Active	1.24 (0.97, 1.59)	1.18 (0.91, 1.51)
Fruit & vegetable consumption (≥ Daily vs < Daily)	0.82 (0.70, 0.96)	0.81 (0.69, 0.97)
BMI, (per Kg/m^2)	1.04 (1.02, 1.05)	1.01 (0.99, 1.03)
History of CVD (CHD or Stroke), (Yes vs No)	2.45 (1.98, 3.04)	1.56 (1.25, 1.94)
History of diabetes, (Yes vs No)	2.26 (1.82, 2.81)	1.61 (1.28, 2.01)
History of cancer, (Yes vs No)	2.14 (1.54, 2.98)	1.86 (1.33, 2.60)
History of COPD, (Yes vs No)	1.59 (1.26, 2.00)	1.20 (0.95, 1.52)
Inflammatory markers, (per 1 SD)		
Alpha-1-acid glycoprotein	1.23 (1.15, 1.32)	0.99 (0.90, 1.08)
Interleukin-6	1.48 (1.39, 1.58)	1.22 (1.12, 1.33)
C-reactive protein	1.33 (1.24, 1.43)	1.07 (0.97, 1.18)

[a] All analyses based on 736 deaths among 6545 individuals
SD: Standard Deviation

Table 4: Hazard ratios for the highest versus lowest tertile of inflammatory biomarkers for all-cause, CVD and cancer mortality over a mean follow-up of 16.7 years

	N[a]	No. deaths[a]	**AGP** HR (95% CI)	**IL-6** HR (95% CI)	**CRP** HR (95% CI)
Model 1: Adjusted for age and sex					
All-cause mortality	6545	736	1.43 (1.20, 1.65)	1.93 (1.58, 2.37)	1.52 (1.27, 1.83)
CVD mortality	6541[b]	181	1.64 (1.11, 2.43)	1.96 (1.29, 2.99)	2.00 (1.35, 2.97)
Cancer mortality	6541[b]	347	1.22 (0.94, 1.59)	1.56 (1.18, 2.08)	1.54 (1.18, 2.02)
Model 2: Adjusted for age, sex, SES, BMI, health behaviors, and chronic disease					
All-cause mortality	6545	736	1.12 (0.92, 1.37)	1.58 (1.27, 1.96)	1.22 (0.99, 1.49)
CVD mortality	6541[b]	181	1.08 (0.71, 1.63)	1.36 (0.86, 2.14)	1.36 (0.88, 2.10)
Cancer mortality	6541[b]	347	0.96 (0.72, 1.27)	1. 28 (0.94, 1.73)	1.29 (0.96, 1.73)
Model 3: Adjusted for all covariates and other inflammatory markers					
All-cause mortality	6545	736	1.01 (0.82, 1.26)	1.53 (1.21, 1.93)	1.04 (0.83, 1.32)
CVD mortality	6541[b]	181	0.94 (0.60, 1.47)	1.23 (0.76, 2.02)	1.27 (0.78, 2.08)
Cancer mortality	6541[b]	347	0.85 (0.62, 1.15)	1.19 (0.86, 1.65)	1.29 (0.92, 1.81)

AGP: alpha-1-acid glycoprotein, IL-6: Interleukin-6, CRP: C-reactive protein (CRP).
[a] Numbers of participants and deaths in the analysis, including those in the middle tertile
[b] Four participants with unknown cause of death have been excluded from these analyses

Figure 1: Life expectancy at age 50 in the lowest and highest tertile* of inflammatory biomarkers in men (Part A) and women (Part B)

* The remaining years of life expectancy at age 50 in those in the lowest and highest tertile of each inflammatory biomarker, calculated using the mortality hazard ratio for each tertile compared with the total sample and applied to the England and Wales age-specific mortality rates for 2011-2013.
The bars show 95% confidence limits.

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