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Brief reports

Higher plasma GlycA, a novel pro-inflammatory glycoprotein biomarker, is associated with reduced life expectancy: The PREVEND study

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ABSTRACT

Objective: Elevated circulating levels of pro-inflammatory biomarkers are associated with adverse health effects, but the extent to which enhanced low-grade inflammation influences remaining life expectancy (LE) is uncertain. GlycA is a novel pro-inflammatory marker. We determined effects of GlycA and high sensitivity C-reactive protein (hsCRP) on LE.

Methods: GlycA and hsCRP were determined in 5526 subjects. LE was compared in the upper quartile of both GlycA and hsCRP vs. the respective lower three quartiles combined, adjusted for LE of individuals in the Dutch general population of the same birth cohort and sex.

Results: Median follow up was 8.5 years [interquartile range 7.9–9.0], during which 348 (6.3%) subjects had deceased. LE at the end of follow up was lower in the highest vs. the lower three quartiles of GlycA ($P < .001$) and hsCRP ($P < .001$). Both men as well as women in the highest GlycA quartile had reduced LE vs. the lowest three quartiles combined ($P < .001$ and $P = .02$). For hsCRP, this was only observed in men ($P < .001$) but not in women ($P = .67$).

Conclusions: This population-based cohort study demonstrates that higher plasma levels of GlycA were associated with reduced LE in men and women. With regard to hsCRP this only applied to men.

1. Introduction

Glycosylation is one the most common post-translational modifications of proteins and is influenced by many biological processes, including inflammation [1]. GlycA is a novel nuclear magnetic resonance (NMR) spectroscopy measured marker of inflammation, which identifies *N*-acetyl glycan groups on enzymatically glycosylated acute phase proteins (primarily α1-acid glycoprotein, haptoglobin, α1-antitrypsin, α1-antichymotrypsin and transferrin) [2,3]. GlycA concentrations are robustly correlated with those of well described inflammatory biomarkers such as high sensitivity C-reactive protein (hsCRP) [2,4].

Several studies have shown that GlycA is positively correlated with cardiometabolic risk factors such as elevated body mass index (BMI) [5], insulin resistance [6] and components of the metabolic syndrome [7]. Furthermore, GlycA is higher in patients with chronic inflammatory conditions [8]. Elevated levels were found to be associated with incident type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD) in several large prospective studies [9–12]. Notably, all of these associations were independent of traditional risk factors.

Besides being a marker of chronic inflammatory diseases, GlycA is also associated with all-cause and cause specific mortality, independently of established risk factors [13–15]. The study of Lawler et al. showed that cardiovascular disease (CVD), colorectal and lung cancer mortality were all significantly associated with elevated levels of GlycA among initially healthy subjects [15]. Another study showed that higher GlycA levels were associated with increased risk of incident colorectal cancer and colorectal cancer mortality, but not with breast cancer or mortality from other cancers [14].

It is evident that elevated levels of GlycA are related to adverse health effects. Notably however, it is uncertain how this influences remaining life expectancy, an increasingly used approach to determine the overall effect of a certain condition on survival [16–18]. We initiated the present study to determine differences in life expectancy in men and women of the Prevention of Renal and Vascular End Stage Disease (PREVEND) cohort with higher vs. lower levels of GlycA and hsCRP. Our method interrogates mortality against life expectancy as the

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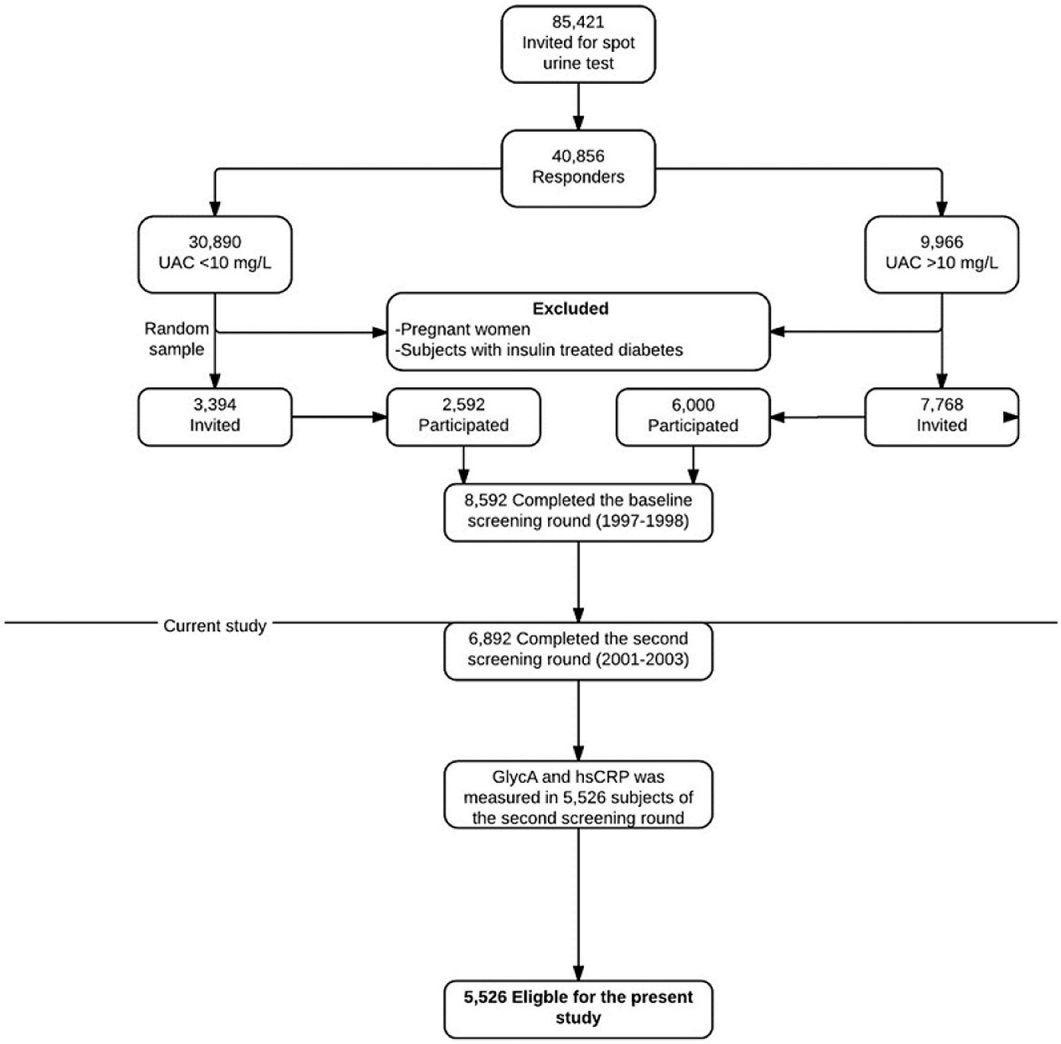

Fig. 1. Flowchart of the study.

time base. As the individual life expectancies at the time of sampling were different, we used left-censoring as well as right-censoring in the calculations of subjects at risk for Kaplan-Meier curves and log-rank statistics.

2. Material and methods

2.1. Study design and population

This study was part of the PREVEND study, a large-scale, observational, general population cohort study bases in the Netherlands. The study began in 1997. Details of the study design and recruitment have been described in previous reports [19,20]. In brief, 40,856 individuals (47.8%) completed a questionnaire on demographics, history of cardiovascular and metabolic outcomes, medication use, and pregnancy before their first visit and collected an early morning urine sample in a vial to measure urinary albumin concentration. Those who were unable or unwilling to participate, individuals using insulin, and pregnant women were excluded. The baseline PREVEND participants were recruited from a total of 6000 individuals with a urinary albumin concentration of 10 mg/L or greater and a random control sample of individuals with a urinary albumin concentration of < 10 mg/L (n = 2592). In total, 8592 individuals constitute the PREVEND cohort and completed an extensive examination between 1997 and 1998. The second screening took place from 2001 through 2003 (n = 6894), which was the starting point of the present evaluation. We excluded 1368 individuals with missing data on GlycA and/or hsCRP, leaving 5526 subjects available for the analyses (Fig. 1).

The PREVEND study was approved by the local medical ethics committee of the University Medical Center Groningen in accord with the Declaration of Helsinki.

2.2. Measurements and laboratory analysis

BMI was calculated as weight (kg) divided by height squared (m^2). Smoking status was defined as self-reported never smoker, former smoker, or current smoker (< 6, 6–20, or > 20 cigarettes/day) and alcohol intake as no/rarely, 1 to 4 drinks/month, 2 to 7 drinks/week, 1 to 3 drinks/day, and 3 or more drinks/day. Blood pressure was measured with an automatic Dinamap XL Model 9300 series device (Johnson-Johnson Medical, Tampa, FL, USA). Hypertension was defined as systolic blood pressure of ≥140 mmHg, a diastolic blood pressure of ≥90 mmHg, or both or the use of antihypertensive agents. Type 2 diabetes mellitus (T2DM) was defined as a fasting serum glucose level ≥ 7.0 mmol/L, a non-fasting plasma glucose level ≥ 11.1 mmol/L, self-report of a physician diagnosis or the use of glucose lowering drugs, retrieved from a central pharmacy registry. Estimated glomerular filtration rate (eGFR) was calculated using the combined creatinine cystatin C-based Chronic Kidney Disease Epidemiology Collaboration equation from 2012 [21].

Table 1
Characteristics of the 5526 subjects of the Prevention of Renal and Vascular End-Stage Disease (PREVEND) study according to sex-stratified quartiles of GlycA.

	Q1	Q2	Q3	Q4	P for trend
Number of subjects	1387	1386	1373	1380	
GlycA, μmol/L					
Men	< 305	≥305	≥339	≥382	
Women	< 314	≥314	≥353	≥394	
Age	49.1 ± 10.8	53.2 ± 12.0	55.4 ± 12.2	56.8 ± 12.1	< 0.001
Sex, n (%)					0.97
Men	655 (47.2)	668 (48.2)	653 (47.6)	657 (47.6)	
Women	732 (52.8)	718 (51.8)	720 (52.4)	723 (52.4)	
BMI, kg/m^2	24.7 ± 3.4	26.2 ± 3.7	27.3 ± 4.2	28.3 ± 4.9	< 0.001
Smoking status, n (%)					< 0.001
Non smoker	541 (39.5)	437 (31.9)	335 (24.7)	311 (22.8)	
Former smoker	588 (42.9)	611 (44.6)	610 (45.0)	539 (39.5)	
Current smoker	242 (17.7)	322 (23.5)	412 (30.4)	513 (37.6)	
Alcohol consumption					0.22
< 10 g/d	1010 (73.4)	990 (72.1)	1004 (73.7)	1033 (75.6)	
≥10 g/d	366 (26.6)	383 (27.9)	358 (26.3)	333 (24.4)	
Hypertension, n (%)	229 (16.5)	417 (30.1)	548 (39.9)	652 (47.2)	< 0.001
Lipid lowering drug use, n (%)	59 (4.3)	111 (8.0)	172 (12.5)	227 (16.4)	< 0.001
History of CVD, n (%)	35 (2.5)	81 (5.8)	98 (7.1)	135 (9.8)	< 0.001
History of cancer, n (%)	82 (5.9)	80 (5.8)	87 (6.3)	89 (6.4)	0.86
T2DM, n (%)	24 (1.7)	51 (3.7)	105 (7.6)	144 (10.4)	< 0.001
Family history of CVD, n (%)	623 (44.9)	677 (48.8)	701 (51.1)	713 (51.7)	0.001
Blood pressure lowering drug use, n (%)	135 (9.7)	264 (19.0)	372 (27.1)	459 (33.3)	< 0.001
Glucose lowering drug use, n (%)	8 (0.6)	28 (2.0)	59 (4.3)	80 (5.8)	< 0.001
SBP, mm Hg	119.0 ± 15.7	124.5 ± 18.2	128.5 ± 19.7	131.3 ± 19.6	< 0.001
DBP, mm Hg	70.4 ± 8.7	72.6 ± 8.8	74.0 ± 8.9	74.7 ± 8.9	< 0.001
Total cholesterol, mmol/L	5.16 ± 1.00	5.40 ± 1.00	5.52 ± 1.05	5.64 ± 1.12	< 0.001
HDL cholesterol, mmol/L	1.34 ± 0.32	1.28 ± 0.30	1.23 ± 0.31	1.20 ± 0.30	< 0.001
Triglycerides, mmol/L	0.85 [0.64–1.18]	1.05 [0.79–1.44]	1.24 [0.90–1.72]	1.39 [1.03–1.87]	< 0.001
eGFR*crea-cysC*, ml/min/1.73m^2	98.6 [88.4–107.7]	94.2 [81.8–104.6]	91.2 [79.0–101.7]	87.5 [75.5–99.7]	< 0.001
UAE, mg/24 h	7.20 [5.68–10.42]	7.83 [5.80–12.27]	8.53 [6.11–14.36]	9.50 [6.28–20.58]	< 0.001

Data are numbers (percentages), means (SD) or medians [interquartile range (IQR)]. P-values were calculated by linear regression analysis or $\chi 2$ analysis. Triglycerides and UAE were logarithmically transformed for analysis. Data with regard to smoking and alcohol consumption were missing in 65(1.2%) and 49 (0.9%) of the subjects, respectively. Abbreviations: BMI: Abbreviations: BMI, body mass index; CVD, cardiovascular disease; DBP, diastolic blood pressure; eGFR*crea-cysC*, estimated glomerular filtration rate based on creatinine-cystatin C equation; HDL, high density lipoproteins; SBP, systolic blood pressure; T2DM, type 2 diabetes mellitus; UAE, urinary albumin excretion.

Fasting blood samples were provided and stored at −80 °C. NMR spectra were collected from EDTA plasma samples using the Vantera® Clinical Analyzer. The GlycA NMR signal is derived from the *N*-acetyl methyl protons of *N*-acetylated carbohydrate side chains of serum glycoproteins (predominantly, α1-acid glycoprotein, haptoglobin, α1-antitrypsin, α1-antichymotrypsin and transferrin) [2]. The GlycA NMR signal is centered at 2.00 ± 0.01 ppm in the NMR spectra of plasma, and only *N*-acetylglucosamine with specific glycosidic linkage, namely, β (1 > 2) or β (1 > 6) with a preceding mannose residue, contribute to the GlycA signal [2].

hsCRP was measured by nephelometry with a threshold of 0.18 mg/L (BNII, Dade Behring). Plasma glucose was measured as described [6]. Serum total cholesterol was assayed on an automatic analyzer type MEGA (Merck, Darmstadt, Germany) using the CHOD-PAP-method. Triglycerides (TG) and high density lipoprotein cholesterol (HDL−C) were measured on a Beckman Coulter AU Analyzer.

The intra-assay and inter-assay coefficients of variation for hsCRP and GlycA are 3% and 4.5%, and 2% and 3%, respectively.

2.3. Statistical analysis

Baseline characteristics are presented according to sex-specific GlycA and hsCRP quartiles. Continuous data are presented as mean with SD or as median with interquartile range (IQR) in case of skewed distribution. Categorical data are presented as numbers with percentages. Pearson correlation coefficients were calculated between GlycA and logarithmically transformed hsCRP. Life expectancy was calculated as follows. The median residual life time (EXP) was derived from gender specific mortality reports provided by the Dutch Central Office of Statistics (CBS) (http://www.CBS.nl). The starting point was the date of blood collection of the individuals at the second screening round.. For any person this date was substituted by the median life expectancy of individuals in the general population with the same age, gender and year of birth. This standardization of survival time enables us to compare mortality at the same life expectancy, thereby omitting influence of other risk factors due to age. Because individual life expectancies at start of follow-up were different, we had to use left-censoring (apart from right censoring) in the calculation of number of persons at risk for Kaplan-Meier curves and log-rank statistics. The time base used in the graphs is negative life expectancy (-EXP). Differences were tested using the log-rank test with left and right censoring. Results were calculated for the whole group and for men and women separately. Life expectancy was compared in the upper quartiles of GlycA and hsCRP vs. the lower three quartiles combined. This cut-off was chosen prior to the analyses to be able to evaluate the effect of higher GlycA and hsCRP. Two-sided P-values < .05 were considered statistically significant. Analyses were performed using SPSS statistics for Windows, Version 23.0 (Armonk, NY: IBM Corp) and Microsoft Excel 2010 for Windows.

3. Results

Baseline characteristics of the 5526 subjects according to sex specific quartiles of GlycA are presented in Table 1. The mean age of the subjects at start of the study was 53.6 ± 12.1 years. 324 (5.9%) of the subjects had T2DM and 349 (6.3%) had a history of CVD. Subjects in the highest quartile of GlycA were older. Levels of total cholesterol, triglycerides and UAE were higher, whereas HDL cholesterol and eGFR were lower in subjects in the highest quartile vs. subjects in the lowest quartile. Hypertension, T2DM and a history of CVD were more prevalent among subjects in the highest quartile. Baseline characteristics

Table 2
Characteristics of the 5526 subjects of the Prevention of Renal and Vascular End-Stage Disease (PREVEND) study according to sex-stratified quartiles of hsCRP.

	Q1	Q2	Q3	Q4	P-value
Number of subjects	1387	1386	1373	1380	
hsCRP, mg/L					
Men	< 0.62	≥0.62	≥1.31	≥2.82	
Women	< 0.64	≥0.64	≥1.43	≥3.33	
Age	49.1 ± 10.8	53.2 ± 12.0	55.4 ± 12.2	56.8 ± 12.1	< 0.001
Sex, n (%)					
Men	682	683	682	683	
Women	744	745	744	745	
BMI, kg/m^2	24.2 ± 3.1	26.2 ± 3.5	27.5 ± 4.0	28.6 ± 5.0	< 0.001
Smoking status, n (%)					< 0.001
Non smoker	521 (37.8)	425 (31.2)	355 (26.1)	323 (23.8)	
Former smoker	535 (38.8)	611 (44.8)	625 (45.9)	577 (42.6)	
Current smoker	323 (23.4)	328 (24.0)	382 (28.0)	456 33.6(	
Alcohol consumption					0.23
< 10 g/d	1004 (72.5)	998 (73.0)	1004 (73.6)	1031 (75.8)	
≥10 g/d	381 (27.5)	369 (27.0)	360 (26.4)	330 (24.2)	
Hypertension, n (%)	255 (18.2)	391 (28.4)	541 (39.4)	659 (47.9)	< 0.001
Lipid lowering drug use, n (%)	93 (6.7)	125 (9.1)	175 (12.7)	176 (12.8)	< 0.001
History of CVD, n (%)	48 (3.4)	68 (4.9)	88 (6.4)	145 (10.5)	< 0.001
History of cancer, n (%)	86 (6.2)	79 (5.7)	93 (6.8)	80 (5.8)	0.66
T2DM, n (%)	34 (2.4)	51(3.7)	95 (6.9)	144 (10.5)	< 0.001
Family history of CVD, n (%)	667 (47.7)	683 (49.5)	680 (49.5)	684 (49.7)	0.68
Blood pressure lowering drug use, n (%)	148 (10.6)	237 (17.2)	373 (27.1)	472 (34.3)	< 0.001
Glucose lowering drug use, n (%)	16 (1.1)	30 (2.2)	53 (3.9)	76 (5.5)	< 0.001
SBP, mm Hg	119 ± 15	125 ± 18	129 ± 19	131 ± 21	< 0.001
DBP, mm Hg	70 ± 9	73 ± 9	74 ± 9	75 ± 9	< 0.001
Total cholesterol, mmol/L	5.18 ± 1.00	5.45 ± 1.04	5.60 ± 1.09	5.50 ± 1.05	< 0.001
HDL cholesterol, mmol/L	1.34 ± 0.31	1.29 ± 0.32	1.24 ± 0.30	1.19 ± 0.29	< 0.001
Triglycerides, mmol/L	0.89 [0.66–1.25]	1.06 [0.78–1.52]	1.24 [0.90–1.74]	1.31 [0.96–1.76]	< 0.001
eGFR*crea-cysC*, ml/min/1.73m^2	99.7 [89.5–108.5]	94.2 [82.6–104.0]	89.8 [67.6–100.4]	88.1 [74.6–100.16]	< 0.001
UAE, mg/24 h	7.10 [5.66–10.20]	7.90 [5.88–1.62]	8.45 [5.93–14.96]	9.70 [6.37–19.85]	< 0.001

Data are numbers (percentages), means (SD) or medians [interquartile range (IQR)]. P-values were calculated by linear regression analysis or $\chi 2$ analysis. Triglycerides and UAE were logarithmically transformed for analysis. Data with regard to smoking and alcohol consumption were missing in 65(1.2%) and 49 (0.9%) of the subjects, respectively. Abbreviations: BMI: Abbreviations: BMI, body mass index; CVD, cardiovascular disease; DBP, diastolic blood pressure; eGFR*crea-cysC*, estimated glomerular filtration rate based on creatinine-cystatin C equation; HDL, high density lipoproteins; hsCRP, high sensitivity C-reactive protein; SBP, systolic blood pressure; T2DM, type 2 diabetes mellitus; UAE, urinary albumin excretion.

according to sex specific quartiles of hsCRP were comparable to those for GlycA (Table 2).

Mean levels of GlycA and median levels of hsCRP of the subjects were 352.3 ± 62.0 μmol/L and 1.36 [0.62–3.08] mg/L, respectively. In the whole group as well as in men and women separately there was a strong positive correlation between GlycA and hsCRP (whole group: $r = 0.67$ P < .001; men: $r = 0.65$, P < .001; women $r = 0.68$, P < .001). Both GlycA and hsCRP were higher in women than in men (357.1 ± 61.4 μmol/L vs.347.0 ± 62.3 μmol/L, P < .001 and 1.41 [0.63–3.25] mg/L vs. 1.31 [0.62–2.84] mg/L, P = .04, respectively).

At the end of a median follow-up of 8.5 [IQR, 7.9–9.0] years, 348 subjects had deceased, of which 74 (21.3%) from CVD, 168 (48.3%) from malignancy and 106 (30.5%) from other causes. Life expectancy was compared in the upper quartiles of GlycA and hsCRP vs. the lower three quartiles combined. At start of the study, subjects in the highest quartile of GlycA were older (Table 1, P < .001). The same was true for subjects in the highest quartile of hsCRP compared to the lowest three quartiles combined (Table 2, P < .001). Life expectancy at the end of follow up was lower in the highest quartile vs. the lower three quartiles of GlycA (P < .001) and hsCRP (P < .001). Fifty percent of the deceased subjects in the highest GlycA quartile had died 1.18 years earlier than expected; this was 0.78 years later for the lowest three GlycA quartiles combined. Of the highest hsCRP quartile 50% had deceased 0.48 years earlier than expected; for the lower three hsCRP quartiles combined this was 1.41 years later (Fig. 2).

Analyses in men (249 deceased subjects) and women (99 deceased subjects) separately showed that life expectancy was significantly different in both men and women in the highest quartile vs. those in the lower quartiles of GlycA (P < .001 and P = .02, respectively). For hsCRP, men in the highest quartile vs. the lower three quartiles had lower life expectancy (P < .001). However, no difference in life expectancy was found when we compared women in the highest quartile vs. women in the lowest three quartiles of hsCRP (P = .67).

4. Discussion

This prospective study comprising 5526 PREVEND study participants demonstrates that higher levels of plasma GlycA and hsCRP are associated with reduced life expectancy. Furthermore, when the analyses were carried out for men and women separately, men as well as women in the highest GlycA quartile had lower life expectancy compared to the lowest three quartiles combined. However, for hsCRP, the association remained only significant in men.

Despite the fact that GlycA and hsCRP are both markers of low-grade systemic inflammation, there are also some noteworthy differences. First, while GlycA is a composite biomarker that integrates both the increased protein levels and enhanced glycosylation states of the most abundant circulating acute phase proteins, hsCRP is a single biomarker of low-grade systemic inflammation. Second, as a result of the foregoing, GlycA has lower intra-individual variability compared to hsCRP and provides a less variable measure of inflammation [2]. Third, hsCRP is an early acute phase protein while the proteins that give rise to the GlycA signal rise later in the acute phase response [22]. Notably, hsCRP circulates at lower concentrations and contributes negligibly to the measured GlycA signal itself [2].

Although this is the first study that investigated the association between GlycA and life expectancy, previous studies have already revealed an association between GlycA and mortality. GlycA was significantly associated with all-cause, CVD, colorectal and lung cancer mortality in 27,524 initially healthy subjects of the Women's Health

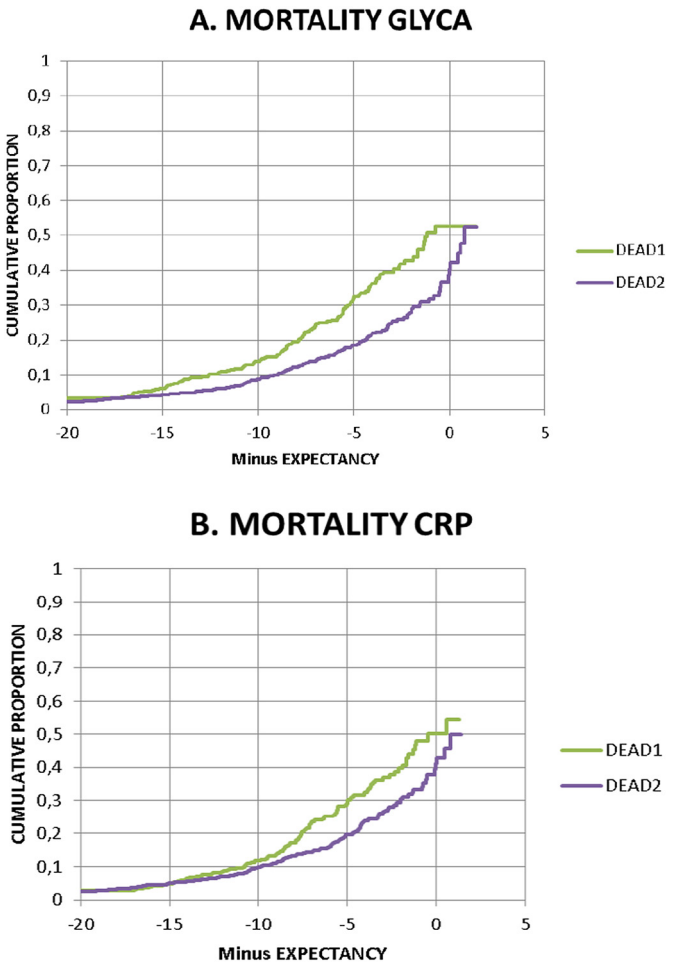

Fig. 2. Kaplan-Meier estimates of overall survival. The survival times were adjusted for sex, age and birth cohort. The time base represents the minus life expectancy in years. The lines show the distribution of the life expectancy at death (n = 348). The green lines show the life expectancy of patients in the highest quartiles of A. GlycA, B. hsCRP ("dead 1"). The purple lines show the life expectancy of subjects in the lower three quartiles combined of A. GlycA and B. hsCRP ("dead 2"). Life expectancy at the end of follow up was lower in the highest quartile vs. the lower three quartiles of GlycA ($P < .001$) and hsCRP ($P < .001$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

study (WHS) [15]. Notably, participants with a history of cancer were not excluded in that report. In addition, the all-cause mortality associations were replicated in an independent cohort of 12,527 subjects of the JUPITER study [15]. The WHS showed an association between GlycA and colorectal cancer (CRC) incidence and mortality. The CRC results were replicated in 6784 men and women from the MESA study [14]. Numerous reports have shown an association between hsCRP and mortality in initially healthy subjects. An overview article of 13 prospective cohort studies of in total nearly 63,000 men and women has shown that elevated hsCRP levels are predictive of near-term and long-term mortality [23]. Additionally, several studies also found an association between elevated hsCRP levels and cardiovascular mortality [24–31].

Remarkably, in the current study hsCRP was only associated with reduced life expectancy in men. Another study in elderly men and women examined whether interleukin-6 (IL-6) and hsCRP were associated with relative survival time and age at death (adult lifespan) [32]. Higher levels of both markers predicted reduced survival time and shorter life span among older men. For women, increased IL-6 levels were associated with lower survival time and shorter lifespan but only in those who were not using estrogens at the time of measurement. Interestingly, in that study hsCRP was again only associated with reduced survival time and lifespan in men but not in women [32]. Although there is no certain explanation for the discrepant results for men and women in our study, it reinforces the idea that GlycA and hsCRP likely capture different aspects of the inflammatory response. Furthermore, due to the low number of deceased females in the highest hsCRP quartile it seems plausible that we did not have enough power to find an association.

Several other methodological considerations need to be discussed. Strengths of this study include the prospective design and considerable follow-up period. The method that we use to examine life expectancy has been demonstrated to give useful information with respect to other disease conditions such as thyroid cancer, and effects of thyroid function status [16,17].

We consider it a strength that it allows to compare survival rates with individuals in the general Dutch population with the same sex and year of birth, enabling to reduce the influences of other risk factors due to age [17]. This study also has certain limitations. First, the cohort predominantly consists of Caucasian individuals. Therefore, our results may not be generalizable to other population. Second, due to the limited number of deaths it was not feasible to conduct cause specific life expectancy analyses with sufficient precision.

In conclusion, this large population based cohort study of men and women demonstrates that higher levels of GlycA and hsCRP were associated with reduced life expectancy. In addition, when the analyses were performed for men and women separately, both men and women in the highest quartiles of GlycA had a lower life expectancy. However, for hsCRP, only men in the highest quartile vs. the lower three quartiles combined had significant lower life expectancy.

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Declaration of interest

MAC is an employee of LabCorp. GlycA measurements were performed by LabCorp (Raleigh, North Carolina, USA) at no cost.

Author contributions statement

EGG and WJS performed statistical analyses. EGG and RPFD wrote the first draft of the manuscript. EGG, MAC, WJS, SJLB and RPFD revised the subsequent drafts. All authors approved the final version of the manuscript.

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