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Jacqueline Roshelli Date

Prediagnostic Plasma Advanced Glycation End-products and Soluble Receptor for Advanced Glycation End-Products and Mortality in Colorectal Cancer Patients

By

Jacqueline Roshelli
Master of Public Health

Epidemiology

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Roberd M. Bostick, MD, MPH
Committee Chair

_____

Veronika Fedirko, PhD, MPH
Committee Member

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By

Jacqueline Roshelli
B.B.A.
University of Georgia
2016

Thesis Committee Chair: Dr. Roberd M. Bostick, MD, MPH

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Abstract

Prediagnostic Plasma Advanced Glycation End-products and Soluble Receptor for Advanced Glycation End-Products and Mortality in Colorectal Cancer Patients

By Jacqueline Roshelli

Advanced glycation end-products (AGEs), formed endogenously or obtained exogenously from diet, may contribute to chronic inflammation, intracellular signaling alterations, and pathogenesis of several chronic diseases including cancer. However, the role of AGEs in cancer survival is less known.

In this study, the associations of pre-diagnostic circulating AGEs and soluble receptor for AGEs (sRAGE) with colorectal cancer (CRC)-specific and overall mortality were estimated using multivariable Cox proportional hazards regression among 1,369 CRC cases in the European Prospective Investigation into Cancer and Nutrition study. Plasma concentrations of AGEs and sRAGE were measured on average 53 months before CRC diagnosis using ultra performance liquid chromatography tandem mass spectrometry.

Over a mean follow-up period of 53 months, 693 deaths occurred of which 541 (78%) were due to CRC. Plasma AGEs, individually or combined, were not statistically significantly associated with CRC-specific or overall mortality. However, a possible interaction by sex was suggested for carboxyethyl lysine (CEL) and CRC-specific mortality, with a positive association observed among women only ($P_{interaction}$=*0.05*). CRC cases with higher sRAGE concentrations were at higher risk of dying from CRC (hazard ratio, $HR_{Q5\ vs\ Q1}$=1.67, 95% CI:1.21-2.30, P_{trend}≤*0.01*) or any cause ($HR_{Q5\ vs\ Q1}$=1.38, 95% CI:1.05-1.83, P_{trend}≤*0.01*). These associations tended to be stronger among cases with type II diabetes.

In conclusion, pre-diagnostic circulating concentrations of AGEs were not associated with CRC-specific and overall mortality in CRC patients. However, a positive association was observed between sRAGE and CRC-specific and overall mortality. Further studies in other settings and exploring potential effect modification by sex and diabetes are needed. Our findings may stimulate further research on AGEs' role in survival among cancer patients.

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Chapter I. Background

Colorectal cancer ("CRC") is cancer that occurs in the colon or rectum. It is the third most common cancer in men and second in women. In 2018, over 1.8 million new cases were diagnosed and there were over 880,000 deaths worldwide.[1] The American Cancer Society estimates 147,950 new cases to be diagnosed and 53,200 new deaths in 2020 in the US alone.[2] Based on 2010 to 2016 SEER data, the overall 5-year relative survival is 64.6% in the U.S. This value varies greatly by tumor stage ranging from 90.2% for localized (confined to the primary site) to 14.3% for distant (metastatic) cancer.[3] Geographically, survival rates are higher in the USA than in Europe possibly due to the timing of diagnosis and adherence to evidence-based guidelines for treatment.[4] Incidence rates appear to closely align with economic development leading to higher rates in developed areas such as Europe, Australia, and the United States.[5]

Molecular Basis for CRC and Clinical Characteristics

CRC begins most often with a series of histological, morphological, and genetic changes to the epithelium leading in most cases to adenomatous polyps.[6] Polyps are protrusions from the mucosal surface which are very common. In colon and rectal cancer, the most common polyps include nonneoplastic hamartoma, hyperplastic mucosal proliferation, and adenomatous polyps. Adenomatous polyps are the only ones that are clearly premalignant. Most adenomatous polyps are benign, but a very small percentage, around 5%, with sufficient genetic changes progress to cancer and acquire the ability to invade the bowel wall.[7] Further, CRC can spread to local lymph nodes and metastasize to distant sites.[8] On a molecular level, for a polyp to turn into CRC, several changes and mutations must occur. There are three major molecular pathways in CRC development: the chromosomal instability ("CIN"), microsatellite instability ("MSI"), and CpG Island Methylator Phenotype ("CIMP") pathways.[9] The CIN pathway begins with mutations in the *APC* gene, impacting chromosome segregation during cell division, then mutations in the *KRAS* oncogene, impacting cell growth, motility, and survival. These changes then

lead to loss of function of the *p53* gene that regulates transcription and apoptosis which can affect many cellular functions and lead to carcinogenesis. The MSI pathway involves disruption of the DNA repair genes leading to uneven replication of repetitive DNA sequences. This disruption creates susceptibility to further genetic mutations. Last, the CIMP pathway starts with mutations in the *BRAF* gene, altering growth signaling and apoptosis. Then, the acquisition of *KRAS* mutations leads to aberrant gene promoter region hypermethylation that results in gene deactivations impacting a range of genes including those responsible for the regulation of cell growth.[8]

Clinically, CRC is detected through regular screenings or symptoms including blood in stool, stomach ache and cramps, or unexplained weight loss. When polyps are discovered in a regular screening, they can be surgically removed to reduce the risk of CRC. Polyps develop in three main areas, the proximal colon, distal colon, or rectum. The most common area is the proximal colon.

Risk Factors for CRC

Several risk factors for CRC have been identified. According to the World Cancer Research Fund and American Institute for Cancer Research, there is strong convincing evidence that physical activity is associated with lower risk of CRC, and processed meat, alcoholic drinks, body fatness, and adult attained height are associated with higher risk of CRC. Additionally, there is strong probable evidence that whole grains, foods containing dietary fiber, dairy products, and calcium supplements are associated with lower risk of CRC and red meat with higher risk of CRC. There is also limited suggestive evidence that vitamin D and fish intake are associated with lower risk of CRC while low intakes of fruits and vegetables with higher risk of CRC.[10] Regionally, incidence rates are higher in developed countries due to a correlation between Western lifestyle and diet and CRC risk.[11,12] The evidence surrounding the many modifiable risk factors for CRC shows that CRC is preventable. However, not as much information is known for CRC survivors and more research is needed in understanding what modifiable factors may improve survival and outcomes in this population.

Risk Factors Associated with Survival After CRC Diagnosis

Several risk factors have been investigated for their association with survival after CRC diagnosis including but not limited to stage at diagnosis, treatment, dietary, and lifestyle factors. For clinical prognosis, tumor stage information is the only source used. Treatment also impacts survival with the current treatment including a combination of chemotherapy and radiation along with surgical removal of the tumor.[13] How specific potentially modifiable dietary and lifestyle factors including but not limited to obesity, physical activity, and healthy dietary patterns may contribute to survival after CRC diagnosis is less clear.[14,15] Research into the role of these modifiable factors in cancer survival is of great public health importance. However, there are several challenges associated with conducting research among cancer survivors – selecting the most relevant timing to capture exposure data (before, at, or after diagnosis) and finding a cancer survivor cohort with detailed diet and lifestyle information, molecular subtypes of tumors, or detailed information on treatment. It is crucial to collect this information by establishing well-designed cancer survivors' prospective cohorts in order to develop guidance specifically tailored for this population.

Advanced Glycation End Products ("AGEs")

AGEs are proteins or lipids that become glycated when they come in contact with sugars.[16] When glucose or other reducing sugars react with amino groups in proteins, lipids, or nucleic acids, they create a series of reactions. The reactions create Schiff bases, compounds with a general structure of $R_1R_2C=NR'$, and Amadori products. These products then lead to the accumulation of reactive intermediates such as dicarbonyl compounds and continue to form AGEs. AGEs crosslink proteins resulting in structural and functional changes and build-up of AGEs in the tissue.[16-18] The accumulation of AGEs through life can lead to intracellular signaling alterations, low-level inflammation, and a decrease in tissue functionality. AGEs are produced exogenously and endogenously.[19] The exogenous formation of AGEs occurs in foods processed at high temperatures, especially meats and sugary products. When the

food is consumed, internal AGEs concentrations are increased. Endogenously, AGEs are formed as a normal by-product of metabolism, but their formation can be accelerated by diabetes, tobacco smoking, and other conditions or actions which result in hyperglycemia.[20-22]

As AGEs are being consumed from diet and endogenously formed in the body, they bind with the receptor for AGEs ("RAGE"). RAGE has an extracellular domain, a transmembrane domain, and a cytoplasmic domain. The binding of AGEs to RAGE can lead to acute and chronic inflammation.[23,24] For CRC carcinogenesis, underlying mechanisms such as insulin resistance, energy balance, and chronic inflammation, which promote tumor aggressiveness and poor survival, could be impacted by AGEs and AGEs binding with RAGE.[25-27]

Soluble Receptor for AGEs ("sRAGE")

sRAGE also binds to AGEs but does not have an intracellular tail and transmembrane domain, so its binding with AGEs does not result in the same oxidative stress and inflammation response as when AGEs bind with RAGE. Low sRAGE concentration has been observed in individuals with hypertension, coronary artery disease (CAD), chronic obstructive pulmonary disease (COPD), hyperthyroidism, rheumatic arthritis, and Alzheimer's disease.[23] However, in several studies, high sRAGE concentration has been associated with higher mortality risk in patients with sepsis and type I and type II diabetes.[28-30] As a result, some studies have hypothesized that sRAGE may be an indicator of the ongoing chronic inflammation. This hypothesis could be explained by the generation of sRAGE *via* the proteolytic cleavage of membrane-bound RAGE, which increases circulating concentration of RAGE due to RAGE activation.[31,32]

AGEs and CRC Risk

Limited epidemiologic evidence exists on the association between dietary intake of AGEs and circulating biomarkers of AGEs and CRC risk. A case-cohort study within the Women's Health Initiative ("WHI") study found a suggestive inverse association between concentrations of N(6)-Carboxymethyl

lysine ("CML") -AGE and risk for CRC (multivariable HR $_{Q4\ vs\ Q1}$ = 0.81, 95% CI: 0.52-1.26) among postmenopausal women.[33] A case-control study nested within the European Prospective Investigation into Cancer and Nutrition study (EPIC) cohort found a positive association between circulating glycer-AGEs (AGEs derived from glyceraldehyde rather than the standard Maillard reaction between an aldehyde group and an amino group) and risk of rectal cancer (multivariable OR $_{\text{rectal Q4 } vs\ Q1}$ = 1.90, 95% CI: 1.14-3.19), but not risk of colon cancer (multivariable OR $_{\text{colon Q4 } vs\ Q1}$ = 0.83, 95% CI: 0.57-1.22).[34]

sRAGE and CRC Risk

Two case-cohort studies investigated the association between blood sRAGE concentrations and CRC risk. A case-cohort study within the WHI cohort found that the highest concentration of sRAGE was associated with significantly lower risk for CRC compared to the lowest concentration of sRAGE among postmenopausal women with BMI > 25 kg/m^2 (HR $_{Q4\ vs\ Q1}$ = 0.39, 95% CI: 0.17-0.91).[33] The second study, a prospective case-cohort study nested in a cancer prevention trial, found that higher prediagnostic concentrations of serum sRAGE were associated with lower risk of CRC among male smokers (RR $_{Q5\ vs\ Q1}$ = 0.52, 95% CI: 0.30-0.89).[26]

AGEs and Mortality among Patients with CRC and Other Cancers

Currently, there are no epidemiologic studies that investigated the association between concentrations of AGEs and mortality among patients with CRC. However, several epidemiologic studies investigated the association between circulating or dietary AGEs and other causes of death among patients with breast cancer and other chronic diseases.

Death from breast cancer, CVD, and all-cause mortality have all been studied as endpoints for their association with AGEs in various populations.[29,35-41] Among postmenopausal women diagnosed with breast cancer (n = 2,073) in the WHI, a prospective observational study, the highest tertile of dietary CML-AGE intake after breast cancer diagnosis when compared to the lowest tertile of CML-AGE

intake was statistically significantly associated with higher risk of all-cause (HR = 1.51, 95% CI: 1.17-1.94), CVD (HR = 2.14, 95% CI: 1.19-3.84), and breast cancer mortality (HR = 1.86, 95% CI: 1.19-2.91).[35]

Even though the other five studies were not among cancer survivors, they supported the WHI's study findings.[29,36-39] The Women's Health and Aging Study I, a prospective cohort study (n=559), found that serum CML concentrations were positively associated with CVD mortality (highest *versus* the lowest three quartiles, HR = 1.94, 95% CI: 1.08-3.48) among older community-dwelling women.[36] A prospective cohort study of 85 patients receiving chronic hemodialysis in Australia found that low molecular weight forms of fluorescent AGEs were associated with higher all-cause mortality (HR=1.41, 95%CI: 1.41-6.60).[37] Among a random sample of nondiabetic individuals (n = 1,141) in Kuopio, East Finland, or West Finland, fasting serum AGEs were associated with all-cause (HR = 1.90, 95% CI: 1.16-3.11) and CHD (HR = 6.51, 95% CI: 1.78-23.79) mortality in women, but not in men.[38] Higher circulating plasma pentosidine was an independent predictor of higher all-cause mortality (RR = 1.04, 95% CI: 1.01-1.08) and CVD mortality (RR = 1.03; 95% CI: 1.01-1.06) among chronic kidney disease patients (n = 551) in a prospective cohort study.[39] In a prospective cohort study of 169 individuals with diabetic nephropathy and 170 with persistent normoalbuminuria higher AGEs concentrations were associated with the incidence of fatal and nonfatal CVD (HR = 1.30, 95% CI = 1.03-1.66) and all-cause mortality (HR = 1.27, 95% CI: 1.00-1.62) in individuals with type 1 diabetes.[42]

Three cohort studies did not find a positive association between AGEs and mortality. A prospective cohort, the CARLA study, found a non-statistically significant inverse association between all-cause mortality and plasma AGEs among 1,760 individuals of the general population in Halle, Germany (HR_{men} = 0.93, 95% CI: 0.83-1.05; HR_{women} = 0.88, 95% CI: 0.74-1.05).[43] The other two prospective cohort studies found that AGEs were not statistically significantly associated with all-cause mortality and/or CVD mortality among end-stage renal disease and hemodialysis patients.[44,45]

sRAGE and Mortality Among Patients with CRC and Other Cancers

Currently, there are no published epidemiologic studies that investigated the association between blood concentrations of sRAGE and mortality among patients with CRC. A retrospective cohort study among melanoma cancer patients (n = 402) showed that lower serum sRAGE concentrations are statistically significantly associated with lower survival among 229 stage III/IV patients ($HR_{low\ vs\ high}$= 1.9, 95% CI: 1.2-3.1).[46] The remaining literature on sRAGE and mortality is among populations other than cancer patients.

In a cross-sectional study of renal transplant recipients (n=591), high circulating sRAGE concentrations were associated with lower risk for death ($HR_{Q4\ vs\ Q1}$ = 0.51, 95% CI: 0.26-0.97).[47] However, three other epidemiologic studies and one meta-analysis found a positive association between high circulating sRAGE concentration and high risk of all-cause mortality. Among frail older adults from two population-based cohorts (n = 141), higher sRAGE was associated with higher risk of mortality ($HR_{\text{per unit increment in ln-sRAGE}}$ = 2.72, 95% CI: 1.48-4.99).[48] No statistically significant association with mortality was found among the non-frail group (n = 550) in the same study.[48] The remaining studies were among individuals who already had chronic or acute conditions. Preexisting conditions could be associated with inflammation and resulting in high concentrations of sRAGE. As a result, the findings may not be generalizable to other populations (e.g., cancer survivors or healthy individuals). In a prospective, observational cohort study among hemodialysis and peritoneal dialysis patients (n = 123), higher plasma sRAGE concentration in patients with a greater increase in brain natriuretic peptide concentration was associated with a higher mortality rate.[49] In a prospective cohort study of 169 individuals with type I diabetes, higher sRAGE concentrations were directly associated with all-cause mortality (HR = 1.90, 95% CI: 1.09-3.31).[29] Finally, in a meta-analysis of eight prospective randomized and observational studies (n = 746), high plasma sRAGE was independently associated with higher 90-

day mortality in patients with acute respiratory distress syndrome ($OR_{\text{per one-ln increment}}$ = 1.18, 95% CI: 1.01-1.38).[50]

Given the current gap in the literature, we investigated the association between prediagnostic circulating AGEs and sRAGE and overall and CRC-specific mortality among patients diagnosed with CRC within the context of a large Western European prospective cohort study.

Chapter II. Manuscript

Prediagnostic Plasma Advanced Glycation End-products and Soluble Receptor for Advanced Glycation End-Products and Mortality in Colorectal Cancer Patients

Abstract

Background: Advanced glycation end-products (AGEs), formed endogenously or obtained exogenously from diet, may contribute to chronic inflammation, intracellular signaling alterations, and pathogenesis of several chronic diseases including cancer. However, the role of AGEs in cancer survival is less known.

Methods: The associations of pre-diagnostic circulating AGEs and soluble receptor for AGEs (sRAGE) with colorectal cancer (CRC)-specific and overall mortality were estimated using multivariable Cox proportional hazards regression among 1,369 CRC cases in the European Prospective Investigation into Cancer and Nutrition study. AGEs and sRAGE plasma concentrations were measured on average 53 months before CRC diagnosis using ultra performance liquid chromatography tandem mass spectrometry.

Results: Over a mean follow-up period of 53 months, 693 deaths occurred of which 541 (78%) were due to CRC. Individual and combined AGEs were not statistically significantly associated with CRC-specific or overall mortality. However, a possible interaction by sex was suggested for carboxyethyl lysine (CEL). Participants with high sRAGE concentrations were at higher risk of dying from CRC (hazard ratio, $HR_{Q5\ vs\ Q1}$=1.67, 95%CI:1.21-2.30, $P_{trend}\leq 0.01$) or any cause ($HR_{Q5\ vs\ Q1}$=1.38, 95%CI:1.05-1.83, $P_{trend}\leq 0.01$). These associations tended to be stronger among cases with type II diabetes.

Conclusion: Pre-diagnostic circulating concentrations of AGEs were not associated with CRC and overall mortality in CRC patients. However, a positive association was observed between sRAGE and CRC and overall mortality. Further studies in other settings and exploring potential effect modification by sex and diabetes are needed.

Impact: Our findings may stimulate further research on AGEs' role in survival among cancer patients.

INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer, and it accounts for around 9% of all cancer deaths worldwide based on 2018 data. With over 1.5 million CRC survivors currently alive only in the US, many individuals are at higher risk for CRC recurrence and death from CRC or other causes.[2] To improve their prognosis, modifiable factors that are associated with improved survival need to be identified. Some observational studies suggest that obesity, smoking, physical inactivity, and low adherence to the World Cancer Research Fund/American Institute of Cancer Research (WCRF/AICR) guidelines are potentially associated with poorer survival among individuals with CRC. [51-54] Specifically, in countries where modern diets high in sugars, meats, and heavily processed foods are common, dietary factors, diabetes, and insulin resistance have been studied for their role in CRC survival.[25,55-57] These factors have also been shown to promote the formation of advanced glycation end products (AGEs).[17,20,22]

Advanced glycation end products (AGEs) are a heterogeneous group of molecules derived from nonenzymatic reactions between reducing sugars and proteins, lipids, or nucleic acids.[16] During the Maillard reaction, carbonyl groups of glucose, fructose, and ribose react with free amine groups of amino acids, nucleic acids, and lipids creating a nonstable Schiff base. A secondary rearrangement reaction forms an Amadori product then these reactive intermediate products accumulate and go on to form AGEs which crosslink proteins leading to changes in their structure and function and causing an accumulation of AGEs in the tissue.[18]

Circulating AGEs including N(6)-carboxymethyl lysine (CML), carboxyethyl lysine (CEL) and methylglyoxal-derived hydroimidazolone 1 (MG-H1), and the soluble receptor for AGEs (sRAGE) may serve as biomarkers of exposure to an unhealthy lifestyle as certain diets have higher levels of AGEs and certain lifestyle choices facilitate endogenous AGEs production. The exogenous formation of AGEs is increased in foods processed at high temperatures, especially meats and sugary products.[19] Endogenous

formation is a normal-by-product of metabolic processes but occurs at faster rates in the presence of hyperglycemia, a common state in diabetes and tobacco smoking.[20,21,58]

In regard to CRC carcinogenesis, AGEs may play a part in the underlying mechanisms including insulin resistance, energy balance, and chronic inflammation all of which are shown to contribute to colorectal carcinogenesis and could promote tumor aggressiveness and poor survival.[25-27] AGEs elicit biological function through the activation of their receptor (RAGE), found in the tissue, leading to the promotion of acute and chronic inflammation and carcinogenesis.[26] Tissue expression of RAGE is generally low throughout the body, but high RAGE expression has been found in tumors of the colon, breast, brain, prostate, and ovary.[59-61] A recent prospective case-only study nested in the Women's Health Initiative (WHI) showed that a higher post-diagnosis dietary AGEs intake was associated with a higher risk of all-cause, cardiovascular disease (CVD), and breast cancer mortality among postmenopausal women with invasive breast cancer.[35] However, a cohort study in Germany, the CARLA study, found no statistically significant associations between all-cause and CVD mortality and plasma AGEs and sRAGE among the general population in Halle, Germany.[43] The inconsistent results between plasma AGEs and risk of mortality are demonstrated in several other studies that include different non-cancer patient populations, various mortality outcomes and age groups. [36-38,42,44]

sRAGE is the soluble receptor for AGEs. It has similar binding specificity as RAGE, but no intracellular tail and transmembrane domain, and it does not induce inflammation or oxidative stress in the tissue.[59] It acts as a decoy by attenuating the inflammatory effects in tissues. In a case-cohort study within the WHI, sRAGE concentrations were inversely associated with risk of CRC among post-menopausal women with BMI > 25 kg/m^2, but not among women with BMI <25 kg/m^2.[33] Another prospective case-cohort study among Finnish male smokers showed a similar association of serum sRAGE with lower risk of CRC.[26] No previous epidemiologic studies have investigated the association of circulating sRAGE with mortality among CRC patients, but a retrospective case-control study found that

lower sRAGE concentrations were significantly associated with poor survival among melanoma patients.[46] Other studies among individuals with non-cancer chronic diseases or conditions or older adults showed low sRAGE concentrations to be associated with CVD, diabetes, metabolic syndrome, and death.[47,62-64] Previous research also suggests that high sRAGE concentrations may reflect high levels of chronic inflammation.[31]

Therefore, the aim of this study was to investigate the association between pre-diagnostic plasma concentrations of AGEs (CML, CEL, MG-H1), and sRAGE and all-cause and CRC-specific mortality in patients diagnosed with CRC within the context of a large, multicenter prospective cohort, the European Prospective Investigation into Cancer and Nutrition study (EPIC). We also studied various ratios of AGEs and sRAGE to better understand the chemical origin of AGEs and their association with CRC mortality and AGEs role in relation to inflammation and other non-communicable diseases.[23]

METHODS

Study population and data collection

EPIC is a multicenter prospective cohort study designed to investigate the associations between diet, lifestyle, genetic and environmental factors and various types of cancer. Participants were recruited from 23 study centers in 10 European countries (France, Germany, Greece, Italy, the Netherlands, Spain, the United Kingdom, Sweden, Denmark and Norway). The rationale and methods of the EPIC design have been published previously[65,66]. Standardized dietary and lifestyle/personal history questionnaires, anthropometric data, and socio-demographic and standardized lifestyle variables including education, smoking, and physical activity and blood samples were collected from most participants at recruitment, before disease onset or diagnosis. Diet over the previous one year was measured at baseline by validated country-specific dietary questionnaires developed to ensure high compliance and better measures of local dietary habits. Blood samples were stored at the International Agency for Research on Cancer (IARC) at -196^{o}C in liquid nitrogen for all countries except Denmark (-150^{o}C, nitrogen vapor).

Individuals who were eligible for the study were selected from the general population of a specific geographical area, town, or province. Exceptions included the French sub-cohort, which is based on members of the health insurance system or state-school employees, and the Utrecht (Netherlands) sub-cohort, which is based on women who underwent screening for breast cancer. The present analysis is based on participant data from all centers except for Norway (blood samples only recently collected; few CRCs diagnosed after blood donation), Sweden (no available plasma samples) and Greece (excluded due to unforeseen data restriction issues). Written informed consent was provided by all study participants. Ethical approval for the EPIC study was obtained from the review boards of the IARC and local participating centers.

Follow-up for cancer incidence

Incident cancer cases were determined through record linkage with regional cancer registries (Denmark, Italy, the Netherlands, Spain, and the United Kingdom) or through a combination of methods including the use of health insurance records, contacts with cancer and pathology registries, and active follow-up through study subjects and their next-of-kin (France and Germany; complete up to June 2010).

Vital status follow-up

Vital status follow-up was determined through record linkage with regional and/or national mortality registries (Denmark, Italy, the Netherlands, Spain, and the United Kingdom) or active follow-up (France and Germany). Censoring dates for complete follow-up varied amongst countries but were between June 2005 and June 2009 for the United Kingdom, December 2006 and June 2009 for Italy and Spain, December 2006 for Denmark, December 2007 for France, December 2008 for the Netherlands, and December 2009 for Germany. Mortality was coded using the 10th Revision of the International Classification of Diseases, Injuries, and Causes of Death (ICD-10) and the outcome was assigned based on underlying cause of death. Thirty study participants had missing cause of death and were excluded only from the analysis of CRC-specific mortality.

Case ascertainment and selection

Cancer data were coded using the tenth Revision of the International Classification of Diseases and the second revision of the International Classification of Disease for Oncology. CRC cases were selected from participants who developed colon (C18.0-C18.7), rectum (C19-C20), and overlapping or unspecified origin tumors (C18.8-C18.9). Anal cancers (C21) were excluded. CRC is defined as colon and rectal cancer cases. Of 1,380 CRC cases with measurements of CEL, CML, and MG-H1, one was excluded due to stage coded as *in situ,* four cases were removed for having a follow-up time of zero, five non-adenocarcinoma cases were excluded, and one case with an implausible value of CML resulting in 1,369 CRC cases for final AGE analyses. Additionally, only in sRAGE analysis, 23 cases were excluded due to missing sRAGE measurements resulting in 1,339 CRC cases.

Biomarker Measurements

Plasma concentrations of protein-bound CML, CEL and MG-H1 were measured in the laboratory of Prof. C. Schalkwijk (Maastrich University, Netherlands) using Ultra Performance Liquid Chromatography tandem Mass Spectrometer (UPLC-MS/MS) as previously described.[22,67] In brief, protein-bound CML, CEL and MG-H1 were extracted from plasma using butanolic hydrochloric acid and analysed in ESI positive multiple reaction monitoring (MRM) mode. AGEs were quantified by calculating the area ratio of each unlabelled peak area to the corresponding internal standard. The sum of AGEs (ΣAGEs, in nmol/L) was calculated by summing up the circulating concentrations of CML, CEL and MG-H1 for each subject. We further calculated the ratios of the AGEs considering their dicarbonyl intermediates: MGO-derived/GO derived (i.e. CEL+MG-H1 divided by CML) (**Supplementary Figure 1**).[67] We also calculated the ratio of CEL/MG-H1 to assess the influence of the relative abundance of lysine-sourced MGO-derived AGEs (CEL) *versus* arginine-sourced MGO-derived AGEs (MG-H1). The ratio of ΣAGEs to sRAGE (ΣAGEs/sRAGE) was calculated using crude concentrations of the AGEs and sRAGE. Circulating sRAGE concentrations were measured in citrated plasma samples by ELISA (Quantikine, R&D

Systems, MN, USA), following the manufacturer's instructions. Previous studies have reported that sRAGE is stable in plasma over a long period of time. [68] Analyses were run with case-control sets randomized across batches (n=40 batches, with an average of 35 case-control pairs analyzed per batch). Intra- and inter-batch coefficients of variation (CV) were assessed by measuring three different samples used as quality controls in duplicate in each. Mean intra- and inter-batch CVs were 1.25% and 6.0%, respectively. Measurements of glycosylated hemoglobin (HbA1c) were done on erythrocyte hemolysate using the high-performance liquid chromatography method with Bio-Rad variant II instrument at Karolinska University Laboratory, Department of Clinical Chemistry, Karolinska University Hospital, Stockholm, Sweden, and were expressed in U.S. National Glycohemoglobin Standardization Program units and as percentages of hemoglobin.[41]

Statistical Analyses

Death from CRC was the primary endpoint and death from any cause was used as a secondary endpoint. Age of first tumor diagnosis and age at death or censor were used as the two-time interval points for patient follow-up time. Separate categories were created for categorical variables with missing values. To evaluate the association between CML, CEL, MG-H1, sRAGE, the sum of AGEs (ΣAGEs), and selected ratios (ΣAGEs/sRAGE, CEL/MG-H1, (CEL+MG-H1)/CML) concentrations and CRC-specific and overall mortality, Cox proportional hazards models stratified by center and adjusted for age at diagnosis, sex, and tumor stage were used to calculate hazard ratios (HRs) and 95% confidence intervals (CIs). A second Cox PH model additionally controlled for location of primary tumor, smoking status, BMI (kg/m^2), year of diagnosis, and baseline diabetes status. Each biomarker or ratio of interest was examined in quintiles and per one standard deviation increase of the respective biomarker or ratio. In addition, analyses were also run separately men and women. *P*-value for linear trend was calculated with the median value of each CML, CEL, MG-H1, sRAGE, AGEs, CEL/MG-H1, and (CEL+MG-H1)/CML quintile included as a continuous variable in the corresponding models.

The proportional hazards assumption was graphically assessed by estimating "log-log" survival curves and checked for parallelism. In addition, the proportional hazards assumption was verified using goodness of fit test methods. Correlations between Schoenfeld residuals and time dependent variables in the Cox model were evaluated to test for any violations of the proportional hazards assumptions.

To determine the final models, the following *a priori* identified covariates were assessed as potential confounders by evaluating if there was a sizeable change (> 10%) in hazard ratios (HRs) after including the variable in the model: age at diagnosis, sex, tumor stage, grade of tumor differentiation (well, moderately, poorly differentiated, unknown), location of primary tumor (colon or rectum), smoking status (never smoker, former smoker, current smoker, unknown), BMI (kg/m^2), year of diagnosis, type II diabetes, intakes of red and processed meats, total energy, fiber, sugar, dairy, and alcohol, and circulating concentrations of creatine, zinc, C-reactive protein (CRP).[40] These variables were chosen based on previous published evidence showing their associations with CRC incidence or survival and/or blood AGEs or sRAGE concentrations.

Information regarding categorization and harmonization of tumor stage data has been previously published[69]. In short, a four-stage classification was used including localized, metastatic, metastatic regional and metastatic distant. The effect of missing tumor stage information on effect estimates was assessed using several approaches. The first approach reclassified missing tumor stage values into a separate missing category and adjusted for the stage variable in the final model (included in the primary analysis). Second, a sensitivity analysis was conducted by excluding participants with missing stage information and subsequently by assessing how the results were affected by the missing stage information. Finally, an imputation of missing stage values was conducted using the SAS PROC MI procedure [70]. The multiple imputation method was based on available data for the other covariates in the model and assumed that the stage data were missing at random. Additional sensitivity analyses were performed by length of follow-up and time between blood collection and cancer diagnosis.

We explored whether the association between CML, CEL, MG-H1, sRAGE, and risk for CRC-specific is non-linear using non-parametric restricted cubic splines[71,72] fitted to a Cox proportional hazards model using the SAS macro "lgtphcurv9".[73] Tests for non-linearity used the likelihood ratio test, comparing the model with only the linear term to the model with the linear and cubic spline terms.[73] *P*-values of nonlinearity tests from these models were consistent with a linear response (**Supplementary Figures 2-5**).

Subgroup analyses by categories of potentially biologically relevant effect modifiers (sex, age at diagnosis, tumor site, colon subsite, tumor stage, year of diagnosis, BMI, physical activity, smoking status, type II diabetes, intakes of red and processed meats, alcohol, dietary calcium, vegetables and fruits, and categories of circulating HbA1c, glyceraldehyde-derived AGEs (glycer AGEs), CRP) were conducted.[34] Stratified multivariable-adjusted HRs and 95% CIs were reported per one SD increase in CML, CEL, MG-H1, or sRAGE. A cross-product of biomarker as a continuous variable (per 1 SD) and the effect modifier of interest as a categorical variable was included in the model to test for multiplicative statistical interaction; and the likelihood ratios based on the models with and without the interaction terms were used to test for statistical significance.

All statistical tests were conducted using SAS version 9.2 (SAS Institute) and *P*-values of < 0.05 were considered statistically significant.

RESULTS

Characteristics of study participants

The distribution of selected baseline characteristics of CRC cases according to quintiles of plasma CML are shown in **Table 1** and in **Supplementary Tables 1-3** for CEL, MG-H1, and sRAGE. Among 1,369 eligible CRC cases, there were 693 deaths (including deaths from CRC = 541, other malignant neoplasms = 62, several other causes with low frequency in each category = 60, missing cause of death =

30). Due to missing values, twenty-three participants were excluded from sRAGE analysis (N = 1,346). Median follow up time was 103 (interquartile range, IQR: 23- 155) months, and CML, CEL, MG-H1, and sRAGE were measured on average 53 (SD = 33) months before CRC diagnosis.

AGEs and mortality among CRC patients

The results of the multivariable-adjusted Cox proportional hazard models for the associations of CML, CEL, and MG-H1 and CRC-specific and overall mortality are shown in **Table 2**. Higher concentrations of CML, CEL, and MG-H1 were associated with lower CRC-specific mortality and overall mortality, although these observations were not statistically significant except for CEL. For CRC-specific mortality the multivariable-adjusted hazard ratio for one SD increase in CEL concentration for women was 1.19 (95% CI: 1.01—1.40) *versus* the same model for men 0.82 (95% CI: 0.66—1.03, $P_{\text{likelihood ratio}}$ = *0.05*). For CRC-specific morality, the multivariable-adjusted HR for the fifth quintile *versus* the first quintile of CML, CEL, or MG-H1 were ($HR_{Q5\ vs\ Q1}$ = 0.80, 95% CI: 0.55-1.17, P_{trend} = *0.93*), ($HR_{Q5\ vs\ Q1}$ = 0.96, 95% CI: 0.69-1.35, P_{trend} = *0.72*), and ($HR_{Q5\ vs\ Q1}$ = 1.02, 95% CI: 0.75-1.40, P_{trend} = *0.23*), respectively.

sRAGE and mortality among CRC patients

High plasma sRAGE concentrations were positively associated with overall mortality in multivariate analyses ($HR_{Q5\ vs\ Q1}$ = 1.38, 95% CI: 1.05-1.83, P_{trend} = *<0.01*) and CRC-specific mortality ($HR_{Q5\ vs\ Q1}$ = 1.67, 95% CI: 1.21-2.30, P_{trend}=*<0.01*) (**Table 2**). This association was stronger among men ($HR_{Q5\ vs\ Q1}$ = 1.70, 95% CI: 1.06-2.74, P_{trend} = *0.01*) than women ($HR_{Q5\ vs\ Q1}$ = 1.46, 95% CI: 0.89-2.40; P_{trend} = *0.03*) for CRC-specific mortality.

Ratios and mortality among CRC patients

The ratio of AGEs/sRAGE was inversely associated with CRC-specific mortality ($HR_{Q5\ vs\ Q1}$ = 0.65, 95% CI: 0.47-0.90, P_{trend} = *0.02*) **(Table 3)**. This association was stronger among men than women for CRC-specific mortality, for men ($HR_{Q5\ vs\ Q1}$ = 0.59, 95% CI: 0.37-0.95, P_{trend} = *0.04*) *vs* women ($HR_{Q5\ vs\ Q1}$ =

0.81, 95% CI: 0.49-1.33, P_{trend} = *0.49*). For the ratios CEL/MG-H1 and (CEL+MG-H1)/CML, the associations with CRC-specific were largely null (CEL/MG-H1: HR $_{Q5\ vs\ Q1}$ = 0.91, 95% CI: 0.63-1.31, P_{trend} = *0.91*); ([CEL+MG-H1]/CML: HR $_{Q5\ vs\ Q1}$ = 1.06, 95% CI: 0.69-1.62, P_{trend} = *0.26*) **(Table 3)**.

Sensitivity Analyses

After exclusion of cases which occurred during the first two years of follow-up then the first four years of follow-up, the overall findings did not change substantially. Additionally, division of cases by time between blood collection and diagnosis into tertiles did not change findings considerably. The analysis was also run by quintiles excluding participants with prevalent or incident diabetes which also did not change findings considerably.

Stratified analyses

Subgroup analyses showed differences in associations between CML, CEL, MG-H1, sRAGE and CRC-specific mortality (**Figure 1, Figure 2**) across select subcategories of potential *a priori* defined biologically plausible effect modifiers.

When analyzing participants based on sRAGE concentration tertiles and HbA1c levels (pre-diabetes or diabetes), participants in the third sRAGE tertile (≥1,191.65 ng/ml) with pre-diabetic HbA1c levels had 2.05 times the hazard (95% CI: 1.35-3.13) of CRC-specific mortality compared to participants in the first sRAGE tertile with diabetic HbA1c levels (**Figure 3**).

DISCUSSION

In this large prospective study of individuals with CRC, we found that circulating plasma concentrations of AGEs were not associated with CRC and overall mortality. Among subgroup and stratified analyses for AGEs, potential effect modification was observed by sex for CEL. Additionally, there were statistically significant interactions for concentrations of AGEs by prevalent and incident

diabetes (CML, MG-H1), time between blood collection and follow-up (CML, MG-H1), and physical activity (MG-H1). For circulating concentrations of sRAGE, we observed a positive association with CRC and overall mortality for men and women. Potential effect modification by HbA1c concentrations was observed for sRAGE.

We originally hypothesized that AGEs are positively with CRC and all-cause mortality due to AGEs' effects on cell function and prior research on CRC risk and mortality. On a cellular level, AGEs bind to their receptor RAGE generating reactive oxygen species which activate nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase and subsequently nuclear factor-kappa B resulting in increased expression of certain pro-inflammatory genes.[23] The expression of these genes can lead to the initiation and progression of disease including cancer.[24] RAGE activation and overexpression have been associated with the pathogenesis of colitis-associated cancer in the colon and increased tumor cell migration and proliferation.[74-77] The RAGE expression suppression was associated with a decrease in tumor angiogenesis and spread in *in vitro* and *in vivo* models.[78,79] Among postmenopausal women diagnosed with breast cancer in the WHI (n = 2,073), higher dietary AGE intake after breast cancer diagnosis was associated with higher risk of all-cause (HR = 1.51, 95% CI: 1.17-1.94), CVD (HR = 2.14, 95% CI: 1.19-3.84), and breast cancer mortality (HR = 1.86, 95% CI: 1.19-2.91).[35] These findings were opposite from our findings that circulating concentrations of AGEs were not associated with CRC or all-cause mortality.

A potential protective role for AGEs, as suggested by our results, could be explained in a few ways. Their role in tumor formation *versus* tumor progression may vary as demonstrated in a study on AGE-effected collagen and lung cancer cells which showed that elderly patients developed fewer metastatic tumors than younger patients even though they were at a higher risk for incidence. Collagen affected by AGEs reduced the migratory ability of lung cancer cells by lowering efficient cell adhesion and proteolytic degradation of collagen.[80] In our study, we did not see effect modification by age for

CML ($P_{interaction}$= 0.05), CEL ($P_{interaction}$ = 0.78), or MG-H1 ($P_{interaction}$ = 0.27) with overall mortality or CRC-mortality. A similar effect may explain potential differences in AGEs association with CRC risk *versus* mortality. Additional research showed that methylglyoxal, an intermediate in AGE formation, may inhibit tumor growth by reducing cell proliferation through lowered protein synthesis[81,82]. There may also be evidence for the beneficial effect of food-derived AGEs through improved antioxidant capacity as demonstrated by an animal feeding study. Mice were fed a bread crust diet which resulted in a moderate increase in reactive oxygen species and activation of mitogen-activated protein kinases and nuclear factor-kappa B pathways, followed by increased expression of antioxidative enzymes which could protect against future severe oxidative stress.[83] An exception to this potential explanation in our data were the results for CEL concentrations among women, which showed a possible higher risk of overall or CRC-specific mortality with higher concentrations of CEL. Differences in sex hormones might impact this association.[84,85] However, more research needs to be done to further explore this observation.

For sRAGE, we hypothesized that sRAGE is negatively associated with CRC and overall mortality as sRAGE is a soluble decoy receptor for AGEs and does not produce the same inflammatory effects as AGEs binding with RAGE.[59] Additionally, sRAGE was shown to contribute to antiatherosclerosis effects through oxidized LDL (ox-LDL) quenching. Ox-LDL is produced in excess in CRC tissue and is part of several mechanisms closely linked to tumorigenesis.[86]

In one prospective case-cohort study nested within a cancer prevention trial, higher pre-diagnostic concentrations of serum sRAGE concentrations were associated with lower CRC risk among Finnish male smokers (RR $_{Q5\ vs\ Q1}$= 0.65, 95% CI: 0.39-1.07).[87] Among overweight and obese postmenopausal women in a case-cohort study within the WHI, higher sRAGE was associated with significantly lower risk for CRC compared to the lowest levels of sRAGE (HR $_{Q4\ vs\ Q1}$ = 0.39, 95% CI: 0.17-0.91).[33] There were no studies among CRC survivors and only one study to date among cancer patients.

A retrospective case-control study among melanoma cancer patients (n = 402) concluded that lower post-diagnostic serum sRAGE concentrations were statistically significantly associated with lower survival among 229 stage III/IV patients ($HR_{\text{lower } vs \text{ higher}}$ = 1.9, 95% CI: 1.2-3.1).[46] The findings of this study contrasted with our results of a direct association between sRAGE and CRC and overall mortality among CRC survivors, which could be due to differences in study populations and timing of sRAGE measurements.

There is also research that demonstrates that high concentrations of sRAGE may be an indicator of ongoing inflammation which aligns with our present findings.[48,49] However, the studies were not conducted among populations similar to the EPIC cohort so they do not support that the usual risk for mortality would be associated with high sRAGE in the same way that they found among already ill or frail and older individuals. A prospective cohort study of patients with septic shock and survival showed that higher sRAGE concentrations were associated with worse outcomes mediated in part by upregulation of pathways related to IL-1α, IFN-γ, and TNF-α.[28] Among frail older adults from two population-based cohorts (n=141), higher concentrations of sRAGE were associated with a higher risk of mortality ($HR_{\text{per unit increment in ln-sRAGE}}$ = 2.72, 95% CI: 1.48-4.99). However, the association was not found in non-frail individuals, contributing further to the complexity of sRAGE's role in survival.[48]

We also analyzed several ratios of AGEs and sRAGE in this study to further elucidate pathways and trends. The AGE/sRAGE ratio has been recently proposed to be a universal biomarker for disease states irrespective of the high or low concentrations of sRAGE.[23] It considers the ability of sRAGE to counteract the negative effects of AGEs by binding to them, thus, potentially offsetting high circulating concentrations of AGEs.[23] However, in our study, we observed an inverse association between the AGEs/sRAGE ratio and CRC-specific and all-cause mortality. Since CEL and MG-H1 both have common pathways that derive from methylglyoxal, we examined the ratio of CEL/MG-H1. Among men, we saw an

inverse association between CEL/MG-H1 and all-cause mortality. Lastly, we looked at (CEL+MG-H1)/CML to verify whether the balance between methylglyoxal-derived AGEs and glyoxal-derived AGEs is associated with CRC mortality. We observed a direct association between (CEL+MG-H1)/CML and CRC mortality among women.

In the subgroup analyses, the association between high plasma concentrations of CML, CEL, MG-H1, sRAGE and CRC-specific mortality was suggestive of a positive association among individuals with type II diabetes. AGEs are naturally formed under hyperglycemic conditions, which occur in individuals with diabetes. Chronic hyperglycemia leads to glycation and oxidation of proteins and lipids causing the formation of AGEs and disruption of the extracellular matrix. It is possible that among diabetic individuals with elevated AGEs, there is a threshold concentration of AGEs that they are more likely to reach resulting in poorer outcomes in this group.[88,89] Additionally, poor kidney function is associated with AGE accumulation and more common in diabetic individuals since diabetes can damage blood vessels in kidneys that filter waste from the blood. In the comparison of participants based on sRAGE tertiles and HbA1c levels, individuals in the highest sRAGE tertiles who have HbA1c levels classified as pre-diabetic and diabetic may have higher mortality rates due to not fully controlling their diets. Once patients are diagnosed as pre- or diabetic, significant dietary adjustments are recommended, which could help lower AGEs dietary consumption. Lastly, a positive association between MG-H1 and risk of all-cause and CRC-specific mortality was observed among individuals who reported to be physically active at baseline. This subgroup could represent the healthiest participants based on their overall lifestyle factors. Physical activity has been shown to augment antioxidant capacity, potentially impairing the formation of AGEs, and positively influencing glycaemic control leading to reduced glucose availability necessary for AGEs formation.[90-94] This association could also have been observed due to chance.

The strengths of this study included its design as a large prospective study which allowed for stratification by sex and colon site. Additionally, the ability to control for multiple confounders improved the analysis of our results. Accounting for missing information on CRC stage through sensitivity analysis and imputation clarified the impact of missing stage data. Lastly, the measurement of AGEs through biomarkers rather than through questionnaires strengthened the accuracy of the biomarker's measurements.

Our study also had several limitations. First, our population was limited to white Europeans and therefore the results cannot be expanded to a diverse group. Additionally, our study did not capture potential variances in AGEs and sRAGE concentrations among different races. However, within these constraints, our study did capture a wide range of ages and both men and women. Our AGE measurements were taken before cancer diagnosis, and it may have been potentially more informative to have measurements at diagnosis and/or after diagnosis. This timing is also a strength since the AGE concentrations were likely not influenced by the tumor or diet/lifestyle changes post-diagnosis and therefore more reflective of usual exposure to AGEs during tumor formation. Lastly, we were unable to test tissue samples which may be a more stable measure than blood and would contain information on RAGE ligands.

The results of this large observational study in Western European populations suggest that circulating plasma concentrations of AGEs are not associated with CRC and overall mortality. Potential effect modification by HbA1c concentrations, sex, time between blood collection and follow-up, and physical activity may impact this association. Further research is necessary to replicate these findings in different populations, and to better understand the inter-related mechanisms with chronic inflammation, molecular cancer subtypes, insulin resistance, and CRC mortality.

Chapter III. Public Health Implications and Possible Future Directions

Currently, the findings from research on CRC risk and modifiable risk factors are applied to CRC patients with no specific guidelines developed for cancer survivors. This study is the first on CRC mortality and AGEs/sRAGE which can further contribute to our understanding of the association between diet and cancer survival. Along with additional studies, clinicians could be provided with information on modifiable factors to give to CRC patients and survivors. Improved lifestyle and dietary recommendations could lead to improved survival. Previous studies have focused on the association between AGEs, sRAGE, and mortality from CVD, acute respiratory distress syndrome, and other chronic diseases and conditions.

Future studies could further explore the association between AGEs, sRAGE, and all-cause mortality among other cancer survivors. The biological plausibility and evidence are strong and prompt further research to better understand the role of AGEs and sRAGE in tumor progression. The EPIC cohort is primarily white Western Europeans, so it would be of interest to conduct similar analyses in a more diverse group. Additionally, studies with tissue samples to measure tissue-specific expression of RAGE, and studies of AGEs concentrations at different time points before, at and after cancer diagnosis may help provide further guidance for this area of research. Correspondingly, future studies may look at molecular subtypes of cancer to see if AGEs are more strongly associated with varying subtypes. Furthermore, studies to improve methods for detecting AGEs and its metabolites could help this area of research as the metabolism is complex for these compounds. Finally, future studies could focus on understanding the inter-related mechanisms with insulin resistance, chronic inflammation, and CRC mortality.

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Figures and Tables

TABLE 1. Selected baseline characteristics of CRC cases (N = 1,369) according to quintiles of circulating N(6)-Carboxymethyllysine (CML) in the EPIC study.

Baseline characteristic	N(6)-Carboxymethyllysine (CML), nmol/L					
	Quintile 1: <=1975 N=273	**Quintile 2: 1976-2286 N = 274**	**Quintile 3: 2287-2648 N = 274**	**Quintile 4: 2649-3218 N = 274**	**Quintile 5: ≥ 3219 N = 274**	***P*-value**
CML, mean (SD), nmol/L	1711.46 (197.16)	2131.95 (86.96)	2453.19 (100.90)	2899.71 (164.33)	4314.62 (1076.61)	*< 0.01*
Age at diagnosis, mean (SD), y	60.91 (7.89)	61.41 (7.98)	63.77 (7.79)	62.98 (7.42)	63.92 (6.66)	*< 0.01*
Women, N (%)	127 (47)	154 (56)	143 (52)	146 (53)	135 (49)	*0.19*
Tumor stage, N (%)[d]						*0.10*
I	69 (25)	80 (29)	75 (27)	69 (25)	60 (22)	
II	65 (24)	48 (18)	56 (20)	48 (18)	54 (20)	
III	81 (30)	70 (26)	76 (28)	87 (32)	96 (35)	
IV	31 (11.4)	39 (14.2)	23 (8.4)	28 (10.2)	36 (13)	
Grade of differentiation, N (%)[d]						*< 0.01*
Well differentiated	27 (9.9)	24 (8.8)	19 (6.9)	16 (5.8)	5 (1.8)	
Moderately differentiated	148 (54)	122 (45)	89 (33)	54 (20)	26 (9.5)	
Poorly differentiated	25 (9.2)	18 (6.6)	30 (11)	22 (8.0)	5 (1.8)	
Undifferentiated	2 (0.7)	1 (0.4)	0 (0)	1 (0.4)	0 (0)	
Location of primary tumor, N (%)						*< 0.01*
Colon	194 (71)	177 (65)	180 (66)	170 (62)	145 (53)	
Rectum	77 (28)	96 (35)	94 (34)	102 (37)	129 (47)	
Year at diagnosis, mean (min-max)	1999 (1993-2010)	1999 (1993-2010)	2000 (1993-2012)	2000 (1994-2008)	2000 (1993-2010)	*0.01*
Smoking status, N (%)[d]						*< 0.01*
Never	108 (40)	116 (42)	112 (41)	125 (46)	106 (39)	
Former	74 (27)	90 (33)	108 (39)	93 (34)	98 (36)	
Current	86 (32)	62 (23)	53 (19)	55 (20)	70 (26)	
BMI, mean (SD), kg/m^2	28.93 (4.75)	26.82 (4.22)	26.53 (4.11)	25.53 (3.66)	25.72 (3.88)	*< 0.01*
Physical activity, N (%)[b, d]						*0.01*
Inactive	44 (16)	36 (13)	39 (14.2)	39 (14.2)	40 (14.6)	
Moderately inactive	79 (29)	66 (24)	82 (30)	67 (24)	88 (32)	
Moderately active	119 (44)	120 (44)	107 (39)	118 (43)	103 (38)	

Active	24 (8.8)	30 (11)	27 (9.9)	21 (7.7)	34 (12.4)	
Dietary calcium, mean (SD), mg/d[a]	939.91 (408.14)	983.81 (459.2)	979.67 (394.64)	1000.7 (401.49)	1067.02 (439.91)	*0.01*
Diabetes, N (%)[c, d]						*0.24*
Yes	38 (14.7)	26 (11)	22 (10.2)	22 (9.9)	19 (8.4)	
No	221 (85)	211 (89)	194 (90)	200 (90)	206 (92)	
Dietary fiber, mean (SD)[a]	21.75 (7.81)	22.58 (7.86)	22.03 (7.54)	23.31 (7.85)	24.61 (8.07)	*<0.01*
Vegetables & fruits, mean (SD)[a]	403.83 (261.49)	401.02 (217.20)	399.61 (230.25)	410.90 (263.93)	375.83 (202.14)	*0.49*
Red & processed meat, mean (SD)[a]	92.19 (49.42)	85.52 (97.46)	84.01 (54.64)	86.32 (57.55)	94.38 (54.47)	*0.26*
Energy intake, mean (SD)[a], kcal	2186.67 (661.19)	2165.09 (928.8)	2116.49 (678.8)	2081.36 (670.24)	2182.53 (611.36)	*0.35*

Abbreviations: SD, standard deviation; N, Number of participants

[a] impute missing value with sex-specific dietary medians.

[b] Sex-specific categories.

[c] This category includes prevalent cases of type II diabetes at baseline and new incident cases identified between baseline and cancer diagnosis.

[d] Total percentages do not add up to 100% because of missing data.

Table 2. Multivariable-adjusted HRs and 95%CIs for CRC-specific and overall mortality according to quintiles of blood AGEs and sRAGE in the EPIC study.

Biomarker Category	Cut-offs[h]	Men		Women		$p_{interaction}$ [d,e,f]	Combined	
		Deaths/ Total	HR (95%)[a,d]	Deaths/ Total	HR (95%)[a,d]		Deaths/ Total	HR (95%)[a,d]
CML, nmol/L								
All-cause Mortality								
Quintile 1	≤ 1975	78/146	1.00 (ref)	60/127	1.00 (ref)		138/273	1.00 (ref)
Quintile 2	[1976-2286]	63/120	0.81 (0.54-1.22)	82/154	1.26 (0.82-1.93)		145/274	0.99 (0.75-1.31)
Quintile 3	[2287-2648]	74/131	0.99 (0.64-1.51)	57/143	0.81 (0.52-1.27)		131/274	0.91 (0.68-1.21)
Quintile 4	[2649-3218]	63/128	0.80 (0.51-1.28)	68/146	0.83 (0.53-1.31)		131/274	0.84 (0.62-1.13)
Quintile 5	≥ 3219	80/139	0.73 (0.45-1.18)	68/135	0.85 (0.51-1.42)	*0.16*	148/274	0.73 (0.53-1.02)
p_{trend}[c]			*0.70*		*0.79*			*0.58*
Per 1 SD[b]		358/664	0.97 (0.84-1.13)	335/705	1.02 (0.86-1.22)	*0.83*	693/1369	0.97 (0.87-1.08)
CRC mortality								
Quintile 1	≤ 1975	58/146	1.00 (ref)	49/126	1.00 (ref)		107/272	1.00 (ref)
Quintile 2	[1976-2286]	46/116	0.77 (0.48-1.24)	64/152	1.09 (0.67-1.76)		110/268	0.95 (0.69-1.31)
Quintile 3	[2287-2648]	55/126	1.14 (0.69-1.87)	48/141	0.83 (0.51-1.37)		103/267	0.98 (0.71-1.36)
Quintile 4	[2649-3218]	52/125	1.08 (0.63-1.85)	52/143	0.69 (0.42-1.16)		104/268	0.90 (0.64-1.27)
Quintile 5	≥ 3219	60/133	0.89 (0.51-1.55)	57/131	0.73 (0.41-1.32)	*0.20*	117/264	0.80 (0.55-1.17)
p_{trend}[c]			*0.83*		*0.96*			*0.93*
Per 1 SD[b]		271/646	1.02 (0.87-1.2)	270/693	1.00 (0.82-1.2)	*0.77*	541/1339	1.01 (0.9-1.13)
CEL, nmol/l								
All-cause Mortality								
Quintile 1	≤ 981	89/144	1.00 (ref)	59/129	1.00 (ref)		148/273	1.00 (ref)
Quintile 2	[982-1241]	73/137	0.58 (0.40-0.83)	64/137	1.02 (0.65-1.61)		137/274	0.72 (0.55-0.94)
Quintile 3	[1242-1476]	74/136	0.66 (0.46-0.96)	60/138	1.03 (0.66-1.62)		134/274	0.80 (0.61-1.05)
Quintile 4	[1477-1785]	61/121	0.66 (0.45-0.96)	80/153	1.20 (0.77-1.87)		141/274	0.89 (0.68-1.16)
Quintile 5	≥ 1785	61/126	0.63 (0.41-0.95)	72/148	1.23 (0.74-2.06)	*0.15*	133/274	0.85 (0.63-1.14)
p_{trend}[c]			*0.01*		*0.10*			*0.44*
Per 1 SD[b]		358/664	0.78 (0.65-0.95)	335/705	1.14 (0.98-1.32)	*0.07*	693/1369	0.96 (0.86-1.07)
CRC mortality								

Quintile 1	≤ 981	68/140	1.00 (ref)	46/126	1.00 (ref)		114/266	1.00 (ref)
Quintile 2	[982-1241]	55/136	0.60 (0.40-0.91)	47/135	1.06 (0.63-1.77)		102/271	0.75 (0.55-1.02)
Quintile 3	[1242-1476]	53/130	0.74 (0.48-1.15)	51/137	1.12 (0.68-1.85)		104/267	0.89 (0.65-1.22)
Quintile 4	[1477-1785]	46/117	0.66 (0.43-1.02)	64/149	1.27 (0.77-2.09)		110/266	0.96 (0.71-1.31)
Quintile 5	≥ 1785	49/123	0.66 (0.41-1.08)	62/146	1.37 (0.77-2.44)	*0.28*	111/269	0.96 (0.69-1.35)
p_{trend}[c]			*0.09*		*0.04*			*0.72*
Per 1 SD[b]		271/646	0.82 (0.66-1.03)	270/693	1.19(1.01-1.40)	*0.05*	541/1339	1.02 (0.91-1.15)
MG-H1, nmol/l								
All-cause Mortality								
Quintile 1	≤ 857	79/145	1.00 (ref)	57/128	1.00 (ref)		136/273	1.00 (ref)
Quintile 2	[858-953]	72/134	1.02 (0.70-1.48)	67/140	0.96 (0.62-1.48)		139/274	1.00 (0.76-1.31)
Quintile 3	[954-1058]	69/123	1.02 (0.69-1.51)	74/151	0.95 (0.61-1.47)		143/274	1.03 (0.79-1.36)
Quintile 4	[1059-1221]	61/126	0.80 (0.54-1.20)	65/148	0.78 (0.50-1.22)		126/274	0.84 (0.63-1.12)
Quintile 5	≥ 1222	77/136	0.88 (0.60-1.30)	72/138	1.04 (0.66-1.64)	*0.91*	149/274	0.97 (0.74-1.29)
p_{trend}[c]			*0.64*		*0.22*			*0.69*
Per 1 SD[b]		358/664	0.97 (0.86-1.10)	335/705	1.09 (0.95-1.26)	*0.38*	693/1369	1.02 (0.93-1.11)
CRC mortality								
Quintile 1	≤ 857	61/143	1.00 (ref)	46/127	1.00 (ref)		107/270	1.00 (ref)
Quintile 2	[858-953]	56/132	1.08 (0.70-1.66)	50/136	0.90 (0.55-1.47)		106/268	1.00 (0.74-1.37)
Quintile 3	[954-1058]	48/117	1.03 (0.64-1.64)	60/149	0.91 (0.56-1.49)		108/266	1.07 (0.78-1.46)
Quintile 4	[1059-1221]	47/124	0.86 (0.54-1.38)	54/147	0.85 (0.52-1.40)		101/271	0.91 (0.66-1.26)
Quintile 5	≥ 1222	59/130	0.90 (0.58-1.41)	60/134	1.05 (0.64-1.75)	*0.83*	119/264	1.02 (0.75-1.40)
p_{trend}[c]			*0.91*		*0.09*			*0.23*
Per 1 SD[b]		271/646	0.99 (0.86-1.14)	270/693	1.15 (0.98-1.35)	*0.31*	541/1339	1.06 (0.96-1.17)
sRAGE, ng/ml[g]								
All-cause Mortality								
Quintile 1	≤ 711	85/173	1.00 (ref)	47/96	1.00 (ref)		132/269	1.00 (ref)
Quintile 2	[712-903]	96/157	1.09 (0.78-1.52)	57/112	1.09 (0.69-1.72)		153/269	1.14 (0.88-1.48)
Quintile 3	[904-1104]	61/129	0.93 (0.64-1.35)	70/141	1.01 (0.64-1.61)		131/270	1.12 (0.85-1.47)
Quintile 4	[1105-1403]	46/92	1.04 (0.69-1.58)	78/177	1.10 (0.71-1.71)		124/269	1.13 (0.86-1.50)
Quintile 5	≥ 1404	54/91	1.33 (0.89-2.00)	83/178	1.40 (0.90-2.19)	*1.00*	137/269	1.38 (1.05-1.83)
p_{trend}[c]			*0.03*		*0.04*			*<0.01*
Per 1 SD[b]		342/642	1.16 (1.02-1.33)	335/704	1.15 (1.00-1.31)		677/1346	1.14 (1.04-1.25)

						0.36		
CRC mortality								
Quintile 1	≤ 711	64/168	1.00 (ref)	36/93	1.00 (ref)		100/261	1.00 (ref)
Quintile 2	[712-903]	70/153	1.12 (0.76-1.67)	41/111	0.93 (0.54-1.59)		111/264	1.10 (0.81-1.49)
Quintile 3	[904-1103]	46/126	0.89 (0.57-1.38)	55/137	1.00 (0.59-1.69)		101/263	1.11 (0.81-1.52)
Quintile 4	[1104-1403]	33/89	1.01 (0.61-1.67)	66/175	1.06 (0.64-1.75)		99/264	1.16 (0.84-1.60)
Quintile 5	≥ 1404	44/88	1.70 (1.06-2.74)	72/176	1.46 (0.89-2.40)	*0.84*	116/264	1.67 (1.21-2.30)
p_{trend}[c]			*0.01*		*0.03*			*<0.01*
Per 1 SD[b]		257/624	1.22 (1.05-1.41)	270/692	1.18 (1.02-1.37)	*0.32*	527/1316	1.21 (1.10-1.33)

Abbreviations: SD, standard deviation; HR, hazard ratio; CRC, colorectal cancer

[a]Quintile 1 was a reference category in each model.

[b]One standard deviation for each biomarker as follows: CML: 1025 nmol/l, CEL: 775 nmol/l, MG-H1: 261 nmol/l, sRAGE: 475 ng/ml

[c]P trend was calculated using the median values and adjusting for all the variables of the corresponding model.

[d]Stratified by center, and adjusted for sex (combined), age at diagnosis (continuous), stage_2016(categorical), location of tumor(categorical), year of DX(continuous), BMI(continuous), smoking status(categorical) and diabetes(categorical).

[e]Used likelihood ratio test here.

[f]Calculated with either quintiles or continuous biomarker.

[g]excluded 23 missing sRAGE

[h]Cutoffs were for overall (men and women combined)

Table 3. Multivariable-adjusted HRs and 95% CIs for CRC-specific and overall mortality according to quintiles of combined circulating AGEs and sRAGE and their ratios in the EPIC study.

		Men		Women			Combined	
Biomarker Category	Cut-offs[h]	Deaths/ Total	HR (95%)[a,d]	Deaths/ Total	HR (95%)[a,d]	$p_{interaction}$[d,e,f]	Deaths/ Total	HR (95%)[a,d]
All AGEs (CML+CEL+MG-H1)[p], nmol/L								
All-cause Mortality								
Quintile 1	≤ 4175	79/137	1.00 (ref)	64/136	1.00 (ref)		143/273	1.00 (ref)
Quintile 2	[4176-4664]	80/146	0.93 (0.64-1.35)	64/128	1.07 (0.69-1.64)		144/274	1.07 (0.82-1.40)
Quintile 3	[4665-5166]	64/134	0.85 (0.57-1.26)	61/140	1.14 (0.74-1.76)		125/274	1.01 (0.76-1.33)
Quintile 4	[5167-6030]	67/125	0.74 (0.50-1.12)	67/149	0.77 (0.50-1.19)		134/274	0.84 (0.63-1.10)
Quintile 5	≥ 6031	68/122	0.79 (0.52-1.20)	79/152	0.94 (0.60-1.49)	*0.98*	147/274	0.89 (0.66-1.19)
p_{trend}[c]			*0.14*		*0.22*			*0.51*
Per 1 SD[b]		358/664	0.90 (0.79-1.04)	335/705	1.10 (0.94-1.29)	*0.41*	693/1369	0.97 (0.88-1.07)
CRC mortality								
Quintile 1	≤ 4175	62/136	1.00 (ref)	49/134	1.00 (ref)		111/270	1.00 (ref)
Quintile 2	[4176-4664]	53/142	0.83 (0.53-1.29)	47/126	0.97 (0.59-1.60)		100/268	1.00 (0.73-1.37)
Quintile 3	[4665-5166]	53/132	0.98 (0.62-1.54)	53/139	1.20 (0.74-1.96)		106/271	1.15 (0.84-1.58)
Quintile 4	[5167-6030]	52/120	0.84 (0.53-1.35)	55/146	0.72 (0.43-1.19)		107/266	0.90 (0.65-1.23)
Quintile 5	≥ 6031	51/116	0.86 (0.53-1.39)	66/148	0.91 (0.54-1.53)	*0.93*	117/264	0.98 (0.70-1.37)
p_{trend}[c]			*0.57*		*0.18*			*0.65*
Per 1 SD[b]		271/646	0.96 (0.82-1.12)	270/693	1.13 (0.95-1.34)	*0.33*	541/1339	1.02 (0.92-1.14)
AGEs (CML+CEL+MG-H1)/sRAGE[g]								
All-cause Mortality								
Quintile 1	≤ 3.60	53/89	1.00 (ref)	80/180	1.00 (ref)		133/269	1.00 (ref)
Quintile 2	[3.61-4.59]	54/109	0.71 (0.46-1.11)	75/160	0.92 (0.62-1.35)		129/269	0.84 (0.64-1.11)
Quintile 3	[4.60-5.58]	72/127	0.68 (0.45-1.02)	74/143	0.97 (0.66-1.43)		146/270	0.86 (0.66-1.13)
Quintile 4	[5.59-7.11]	81/150	0.78 (0.52-1.17)	50/119	0.56 (0.36-0.87)		131/269	0.71 (0.54-0.93)
Quintile 5	≥ 7.12	82/167	0.59 (0.39-0.88)	56/102	0.83 (0.54-1.29)	*0.11*	138/269	0.68 (0.51-0.90)

p_{trend}[c]			*0.02*		*0.52*			*0.02*
Per 1 SD[b]		342/642	0.86 (0.76-0.98)	335/704	0.95 (0.81-1.11)	*0.37*	677/1346	0.89 (0.80-0.98)
CRC mortality								
Quintile 1	≤ 3.60	42/88	1.00 (ref)	65/177	1.00 (ref)		107/265	1.00 (ref)
Quintile 2	[3.61-4.59]	39/103	0.79 (0.47-1.33)	61/160	0.94 (0.61-1.46)		100/263	0.91 (0.67-1.24)
Quintile 3	[4.60-5.58]	52/124	0.67 (0.41-1.08)	61/138	0.98 (0.63-1.51)		113/262	0.86 (0.64-1.17)
Quintile 4	[5.59-7.11]	65/148	0.82 (0.51-1.30)	39/117	0.61 (0.37-0.99)		104/265	0.73 (0.53-0.99)
Quintile 5	≥ 7.12	59/161	0.59 (0.37-0.95)	44/100	0.81 (0.49-1.33)	*0.26*	103/261	0.65 (0.47-0.90)
p_{trend}[c]			*0.04*		*0.49*			*0.02*
Per 1 SD[b]		257/624	0.85 (0.73-0.99)	270/692	0.94 (0.79-1.12)	*0.39*	527/1316	0.88 (0.79-0.98)
CEL/MG-H1								
All-cause Mortality								
Quintile 1	≤ 0.92	92/146	1.00 (ref)	64/127	1.00 (ref)		156/273	1.00 (ref)
Quintile 2	[0.93-1.17]	72/134	0.61 (0.43-0.87)	59/140	0.72 (0.46-1.13)		131/274	0.66 (0.51-0.86)
Quintile 3	[1.18-1.48]	68/129	0.65 (0.45-0.94)	72/145	0.85 (0.53-1.36)		140/274	0.78 (0.60-1.03)
Quintile 4	[1.49-1.83]	69/134	0.54 (0.36-0.79)	65/140	0.88 (0.53-1.44)		134/274	0.72 (0.53-0.96)
Quintile 5	≥ 1.84	57/121	0.66 (0.42-1.04)	75/153	1.02 (0.60-1.74)	*0.72*	132/274	0.87 (0.63-1.20)
p_{trend}[c]			*0.03*		*0.22*			*0.44*
Per 1 SD[b]		358/664	0.80 (0.65-0.97)	335/705	1.10 (0.94-1.28)	*0.14*	693/1369	0.96 (0.86-1.07)
CRC mortality								
Quintile 1	≤ 0.92	69/142	1.00 (ref)	54/125	1.00 (ref)		123/267	1.00 (ref)
Quintile 2	[0.93-1.17]	54/128	0.77 (0.51-1.15)	42/138	0.60 (0.36-1.00)		96/266	0.68 (0.50-0.92)
Quintile 3	[1.18-1.48]	55/127	0.70 (0.45-1.07)	59/141	0.84 (0.50-1.42)		114/268	0.83 (0.61-1.13)
Quintile 4	[1.49-1.83]	51/130	0.58 (0.36-0.92)	51/137	0.88 (0.50-1.54)		102/267	0.79 (0.57-1.11)
Quintile 5	≥ 1.84	42/119	0.68 (0.40-1.17)	64/152	1.00 (0.55-1.81)	*0.64*	106/271	0.91 (0.63-1.31)
p_{trend}[c]			*0.08*		*0.14*			*0.91*
Per 1 SD[b]		271/646	0.80 (0.62-1.03)	270/693	1.13 (0.96-1.33)	*0.08*	541/1339	0.99 (0.88-1.12)
(CEL+MG-H1)/CML								
All-cause Mortality								
Quintile 1	≤ 0.70	86/143	1.00 (ref)	65/130	1.00 (ref)		151/273	1.00 (ref)
Quintile 2	[0.71-0.88]	71/136	0.93 (0.64-1.36)	66/138	0.91 (0.53-1.54)		137/274	1.00 (0.74-1.34)

Quintile 3	[0.89-1.06]	73/129	0.90 (0.60-1.36)	56/145	0.89 (0.49-1.60)		129/274	0.97 (0.70-1.34)
Quintile 4	[1.07-1.29]	70/129	0.89 (0.56-1.41)	75/145	1.15 (0.64-2.07)		145/274	1.19 (0.85-1.67)
Quintile 5	≥ 1.29	58/127	0.71 (0.42-1.20)	73/147	1.02 (0.54-1.90)	*0.64*	131/274	1.02 (0.71-1.47)
p_{trend}[c]			*0.16*		*0.07*			*0.58*
Per 1 SD[b]		358/664	0.87 (0.71-1.06)	335/705	1.16 (0.99-1.35)	*0.20*	693/1369	1.03 (0.92-1.15)
CRC mortality								
Quintile 1	≤ 0.70	67/138	1.00 (ref)	56/129	1.00 (ref)		123/267	1.00 (ref)
Quintile 2	[0.71-0.88]	54/131	0.84 (0.54-1.31)	47/133	0.96 (0.52-1.77)		101/264	0.90 (0.63-1.26)
Quintile 3	[0.89-1.06]	49/124	0.68 (0.41-1.13)	42/141	1.06 (0.53-2.10)		91/265	0.89 (0.60-1.31)
Quintile 4	[1.06-1.29]	56/127	0.75 (0.43-1.30)	60/144	1.31 (0.67-2.57)		116/271	1.16 (0.78-1.72)
Quintile 5	≥ 1.29	45/126	0.59 (0.32-1.11)	65/146	1.49 (0.73-3.05)	*0.60*	110/272	1.06 (0.69-1.62)
p_{trend}[c]			*0.21*		*0.02*			*0.26*
Per 1 SD[b]		271/646	0.86 (0.68-1.09)	270/693	1.24 (1.04-1.47)	*0.11*	541/1339	1.07 (0.95-1.21)

Abbreviations: SD, standard deviation; HR, hazard ratio; CRC, colorectal cancer

[a]Quintile 1 was a reference category in each model.

[b]One standard deviation for each biomarker as follows: AGEs: 1474 nmol/l, AGEs/sRAGE: 2.45, CEL/MG-H1: 0.80, (CEL+MG-H1)/CML: 0.39

[c]P trend was calculated using the median values and adjusting for all the variables of the corresponding model.

[d]Stratified by center, and adjusted for sex (combined), age at diagnosis (continuous), stage_2016(categorical), location of tumor(categorical), year of DX(continuous), BMI(continuous), smoking status(categorical) and diabetes(categorical).

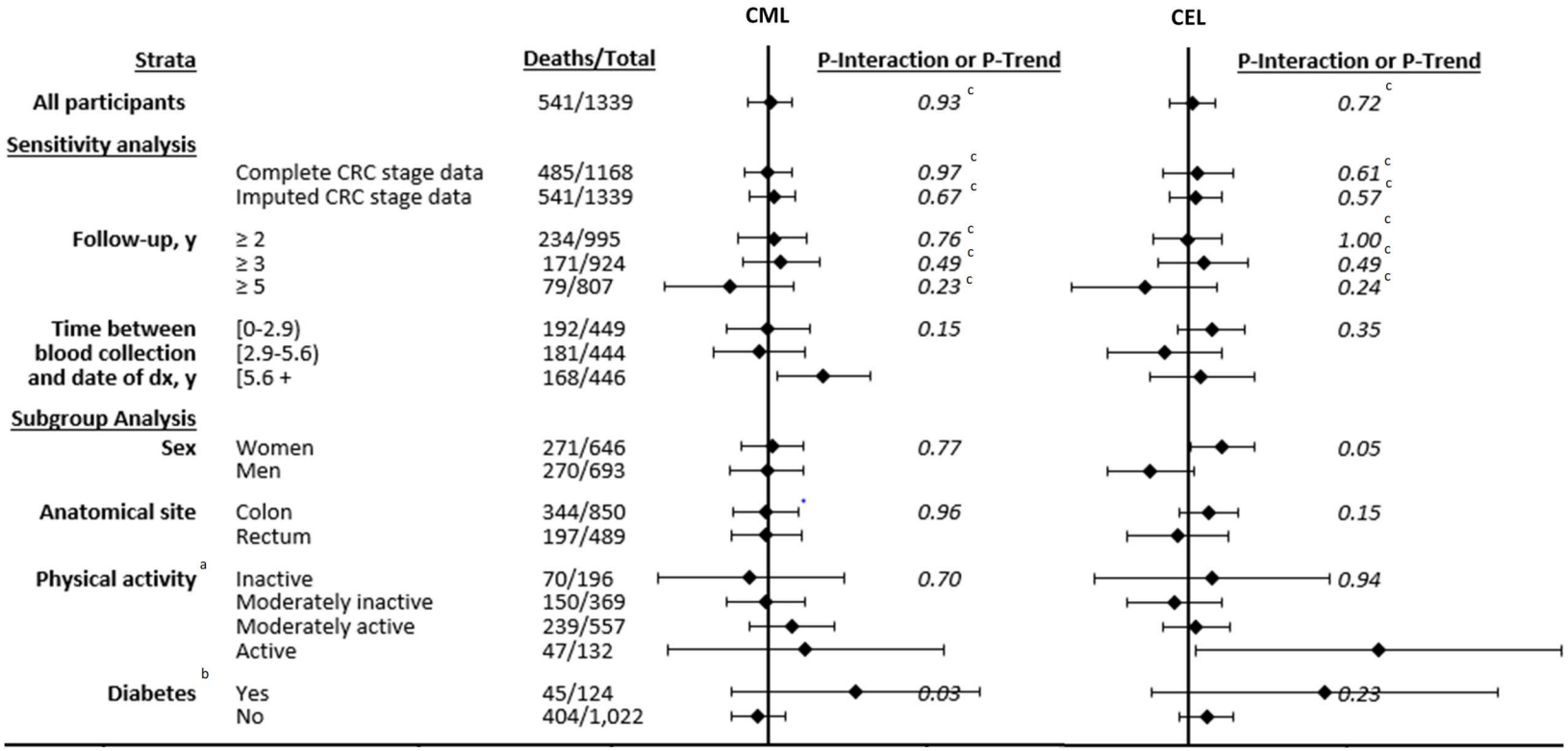
[e]Used likelihood ratio test here.

[f]Calculated with either quintiles or continuous biomarker.

[g]excluded 23 missing sRAGE.

[h]Cutoffs were for overall (men and women combined).

Figure 1. Multivariable-adjusted HRs and 95% CIs for one SD change in CML or CEL for CRC-specific mortality across strata of effect modifiers in the EPIC study.



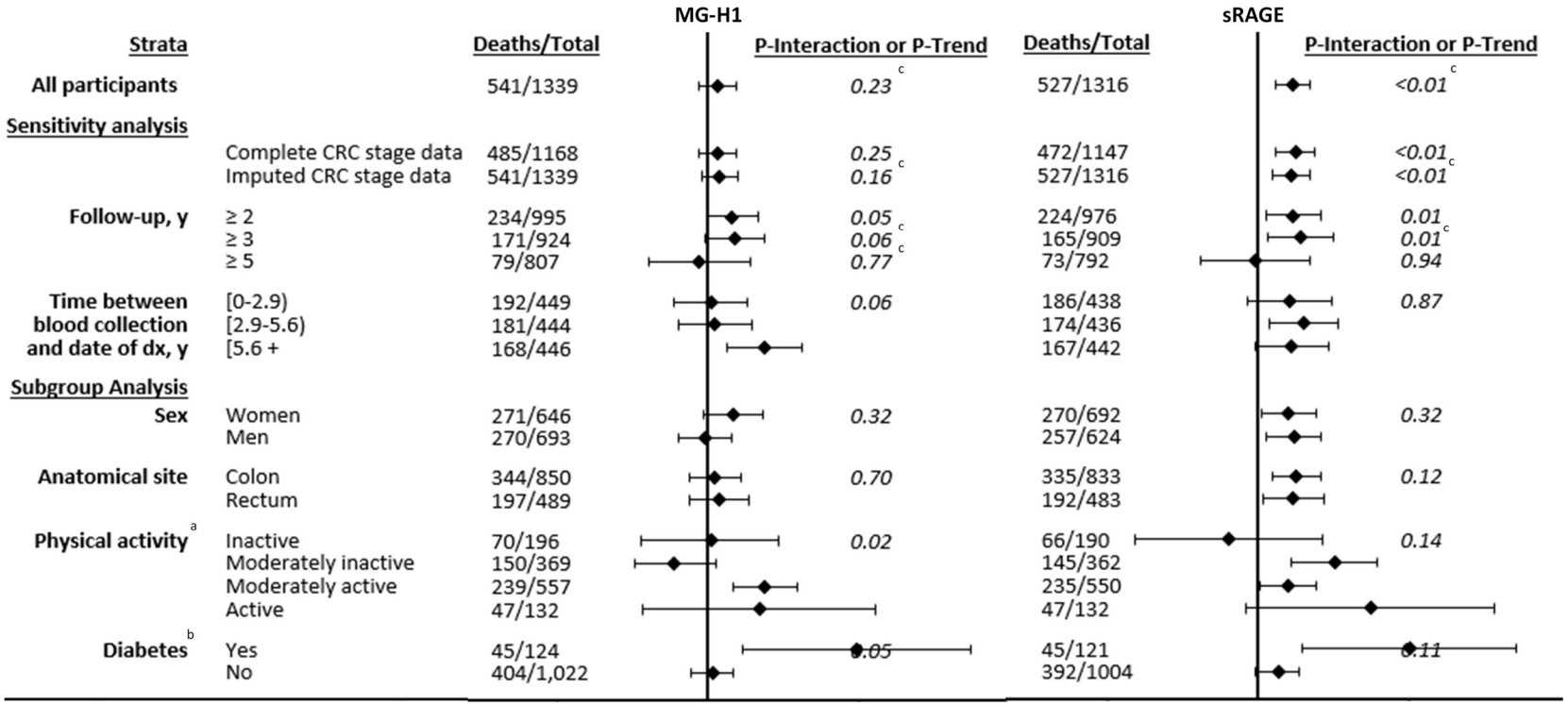
Abbreviations: dx, diagnosis; y, years; CRC, colorectal cancer.

[a] Sex-specific.

[b] This category includes prevalent cases of type II diabetes at baseline and new incident cases identified between baseline and cancer diagnosis.

[c] $p_{trend.}$

Figure 2. Multivariable-adjusted HRs and 95% CIs for one SD change in MG-H1 or sRAGE for CRC-specific mortality across strata of effect modifiers in the EPIC study.



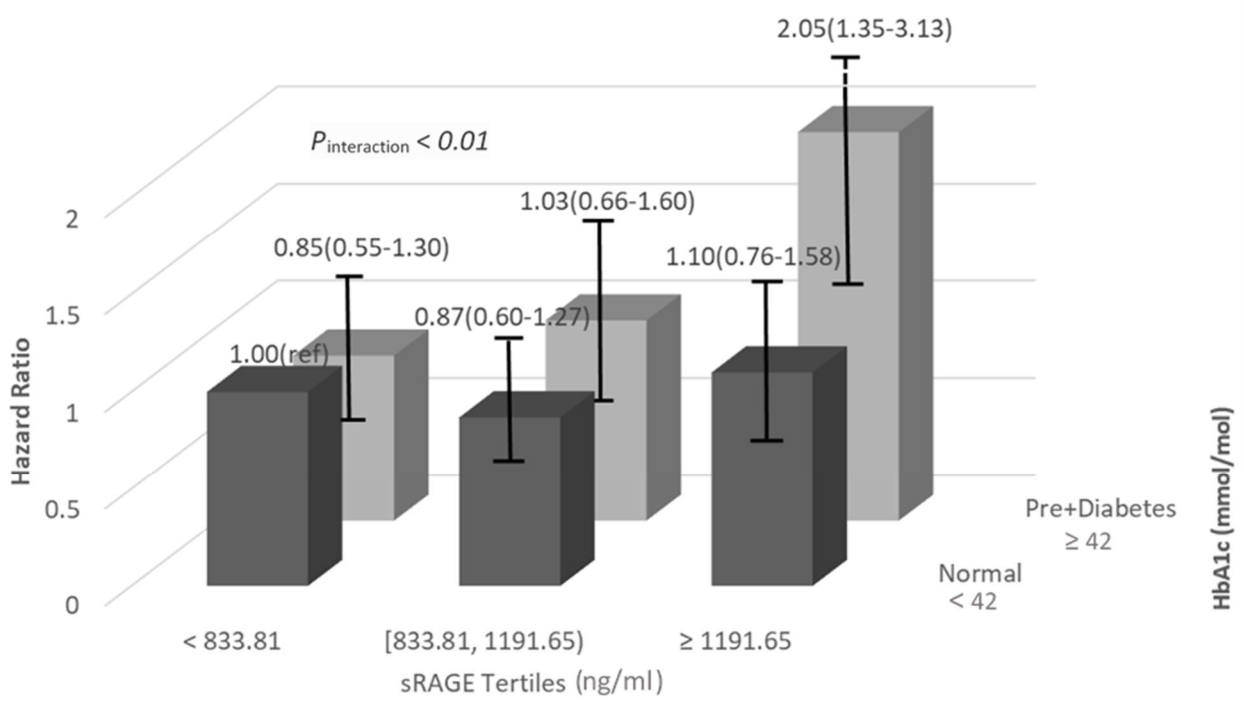
Abbreviations: dx, diagnosis; y, years; CRC, colorectal cancer

[a] Sex-specific

[b] This category includes prevalent cases of type II diabetes at baseline and new incident cases identified between baseline and cancer diagnosis.

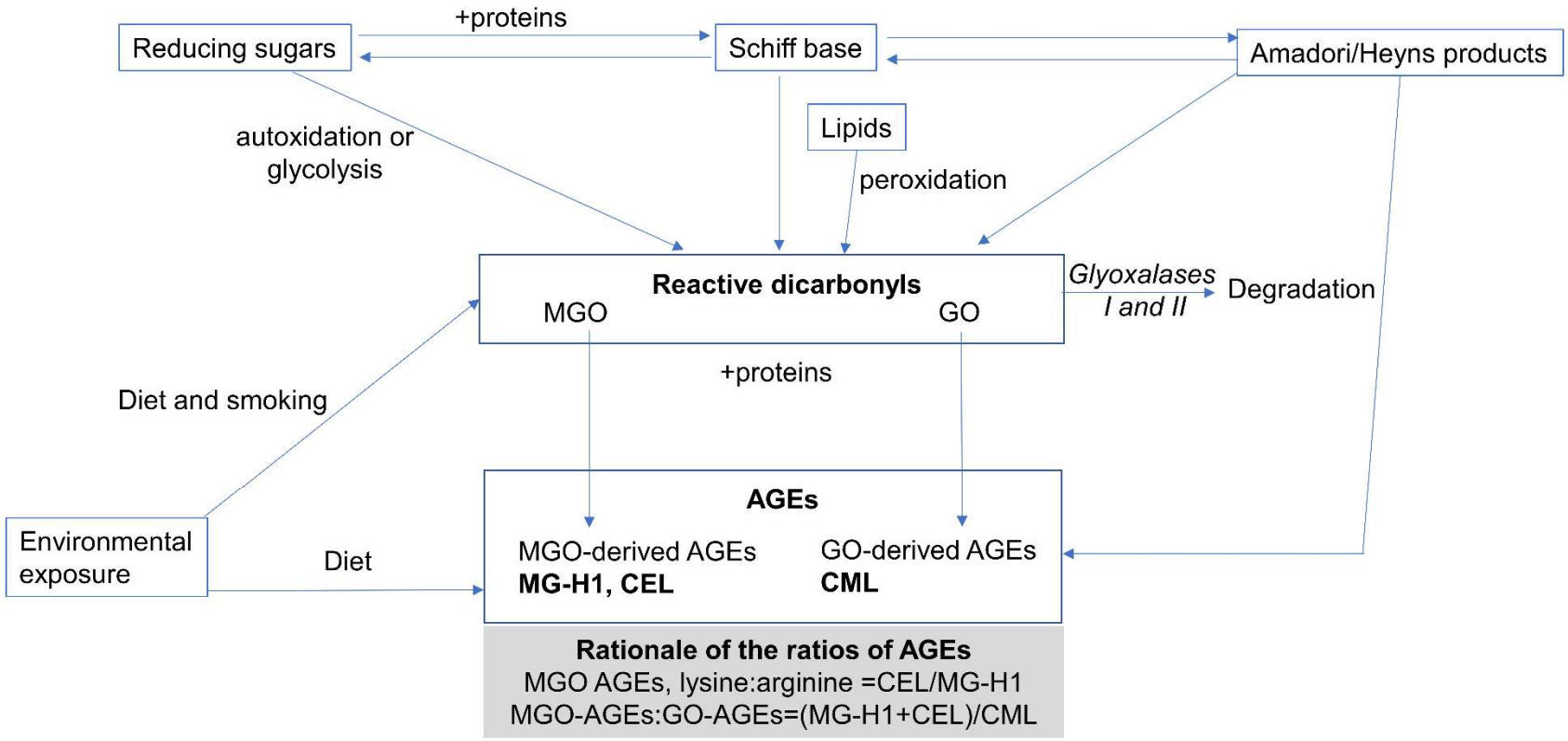
[c] p_{trend}

Figure 3. Multivariable-adjusted HRs and 95% CIs for HbA1c Categories (Normal and Pre-/Diabetes) and sRAGE concentration tertiles and CRC-Specific Mortality in the EPIC study. $P_{interaction}$ <*0.01*.

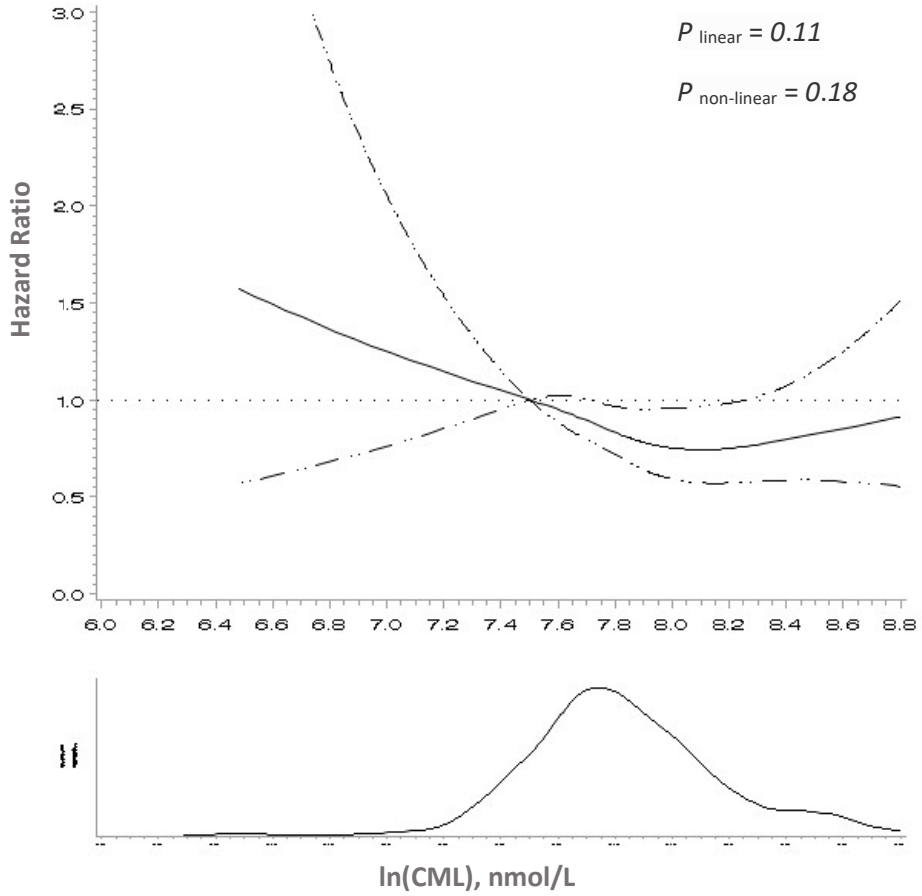


Supplementary Figures

Supplementary Figure 1. Schematic representation of the formation of the AGEs and the rationale for the calculation of the ratios[67]

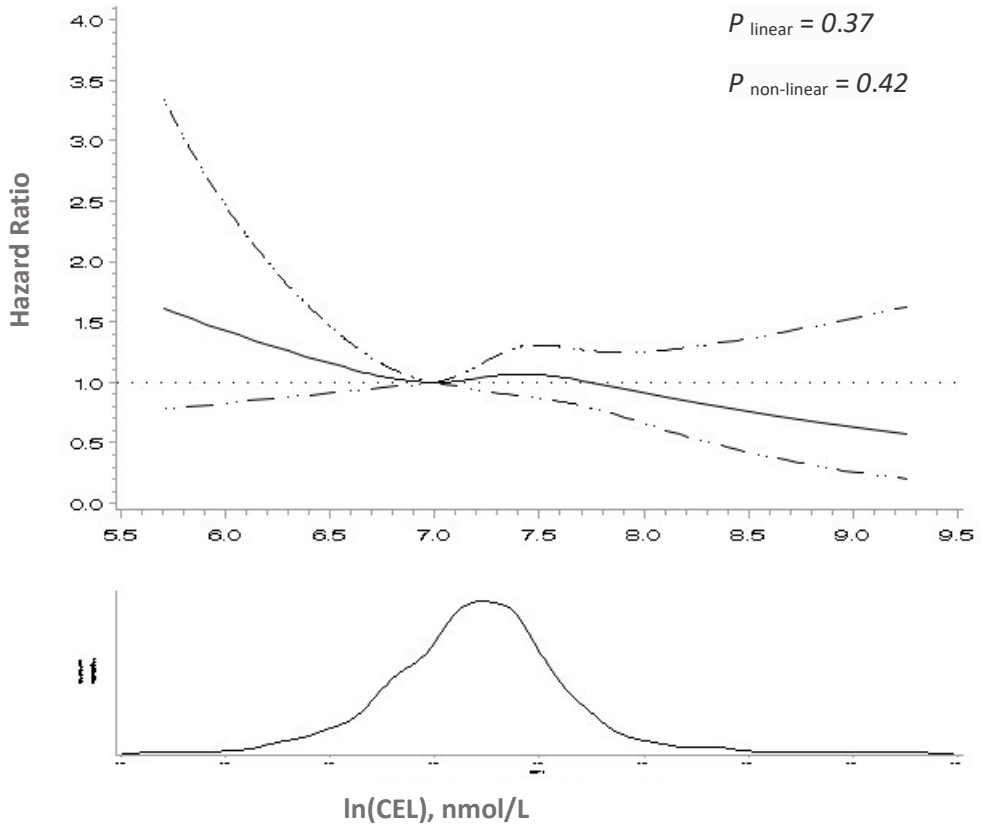


Supplementary Figure 2. Spline regression model for concentration of natural log-transformed CML (nmol/L) and all-cause death. Reference 7.5 nmol/L. *Solid line*- HR, *dashed lines*- 95 % CI.[1]

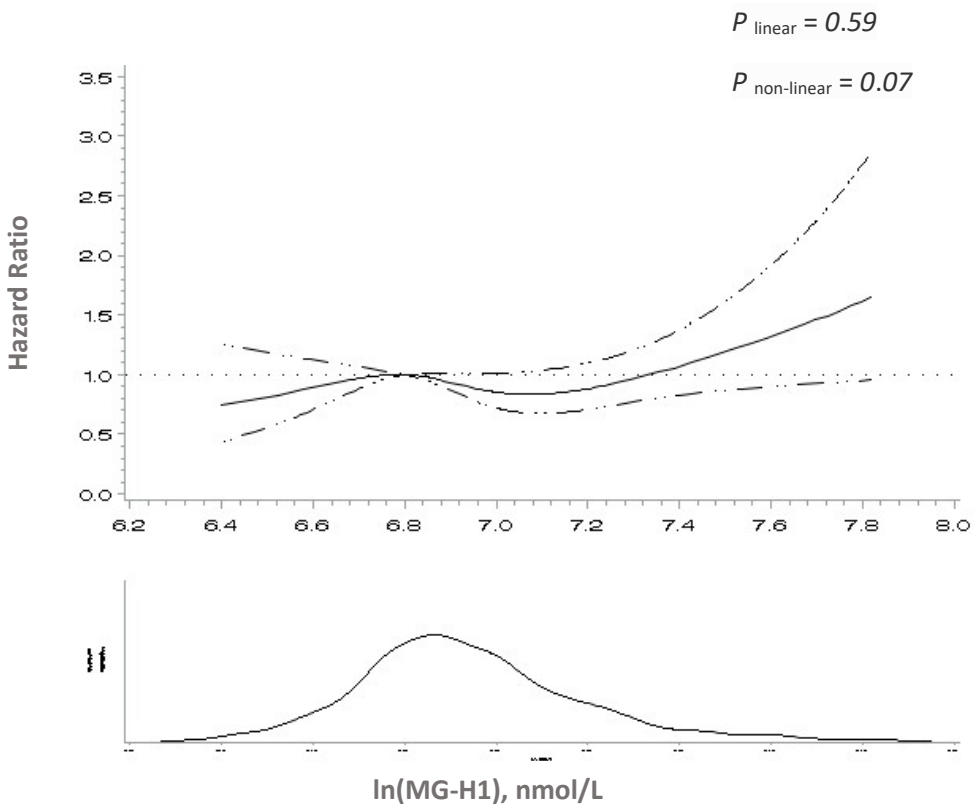


[1] Extreme outliers removed to improve stability of dose response association.

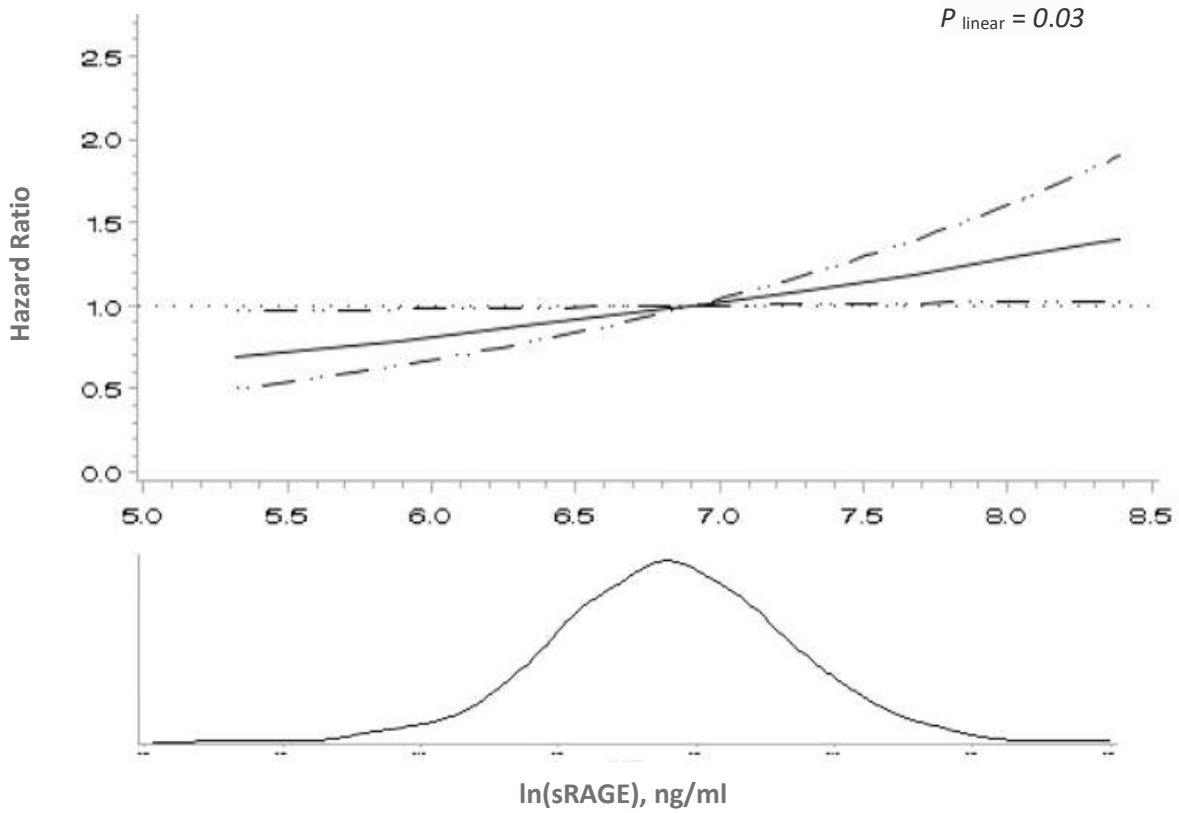
Supplementary Figure 3. Spline regression model for concentration of natural log-transformed CEL (nmol/L) and all-cause death. Reference 7 nmol/L. *Solid line*- HR, *dashed lines*- 95 % CI. $P_{linear} = 0.37$. $P_{non\text{-}linear} = 0.42$.



Supplementary Figure 4. Spline regression model for concentration of natural log-transformed MG-H1 (nmol/L) and all-cause death. Reference 7 nmol/L. *Solid line*- HR, *dashed lines*- 95 % CI. $P_{linear} = 0.59$. $P_{non\text{-}linear} = 0.07$.



Supplementary Figure 5. Spline regression model for concentration of natural log-transformed sRAGE (ng/ml) and all-cause death consistent with a linear association. Reference 7 nmol/L. *Solid line*- HR, *dashed lines*- 95 % CI. $P_{linear} = 0.03$



Supplementary Figure 6. Multivariable-adjusted HRs and 95% CIs for HbA1c Categories (Normal and Pre-/Diabetes) and sRAGE concentration tertiles and overall mortality in the EPIC study. $P_{interaction}$ = *0.01*.