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

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Key Words: advanced glycation end products (AGEs), soluble receptor of AGEs (sRAGE), colorectal cancer, mortality

Abbreviations: AGEs, advanced glycation end products; BMI, body mass index; CEL, Nε-[carboxy-ethyl]lysine; ChREBP, carbohydrate-responsive element-binding protein; CI, confidence interval; CML, Nε-[carboxy-methyl]lysine; CRC, colorectal cancer; CVD, cardiovascular disease; CVs, coefficients of variation; EPIC, European Prospective Investigation into Cancer and Nutrition; GO, glyoxal; HbA1c, glycosylated hemoglobin; HR, hazard ratio; IARC, International Agency for Research on Cancer; ICD-10, 10th Revision of the International Classification of Diseases, Injuries, and Causes of Death; IQR, interquartile range; MG-H1, Nδ-[5-hydro-5-methyl-4-imidazolon-2-yl]-ornithine; MGO, methylglyoxal; N, number of participants; OR, odds ratio; ox-LDL, oxidized low-density lipoprotein; PH, proportional hazard; RAGE, receptor for AGEs; RR, risk ratio; SD, standard deviation; Sp1, specificity protein 1; sRAGE, soluble receptor of AGEs; UPLC-MS/MS, ultra-performance liquid chromatography tandem mass spectrometry; vs., versus; WCRF/AICR, World Cancer Research Fund/American Institute of Cancer Research; WHI, Women's Health Initiative; ΣAGEs, the sum of AGEs.

Category: Cancer Epidemiology

Novelty and Impact: Advanced glycation end-products (AGEs) may contribute to chronic inflammation, intracellular signaling alterations, and pathogenesis of several chronic diseases including colorectal cancer (CRC). However, the role of AGEs and their soluble receptor (sRAGE) in CRC survival is less known. This is the first prospective study to examine the association between AGEs and sRAGE and CRC-specific and all-cause mortality among individuals with CRC. Our findings may promote further research on the role of AGEs and sRAGE in survival among cancer patients.

ABSTRACT

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 colorectal cancer (CRC). However, the role of AGEs in CRC survival is less known. The associations of pre-diagnostic circulating AGEs and their soluble receptor (sRAGE) with CRC-specific and overall mortality were estimated using multivariable-adjusted Cox proportional hazards regression among 1,369 CRC cases in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. Concentrations of major plasma AGEs, N^{ε}-[carboxy-methyl]lysine (CML), N^{ε}-[carboxy-ethyl]lysine (CEL) and N^{δ}-[5-hydro-5-methyl-4-imidazolon-2-yl]-ornithine (MG-H1), were measured using ultra-performance liquid chromatography mass-spectrometry. sRAGE was assessed by enzyme-linked immunosorbent assay. Over a mean follow-up period of 96 months, 693 deaths occurred of which 541 were due to CRC. Individual and combined AGEs were not statistically significantly associated with CRC-specific or overall mortality. However, there was a possible interaction by sex for CEL ($P_{interaction}$=*0.05*). Participants with higher sRAGE had a higher risk of dying from CRC ($HR_{Q5\ vs\ Q1}$=1.67, 95% CI: 1.21-2.30, P_{trend}=0.02) or any cause ($HR_{Q5\ vs\ Q1}$=1.38, 95% CI: 1.05-1.83, P_{trend}=0.09). These associations tended to be stronger among cases with diabetes ($P_{interaction}$ = 0.03) and pre-diabetes ($P_{interaction}$< 0.01) before CRC diagnosis. Pre-diagnostic AGEs were not associated with CRC-specific and overall mortality in individuals with CRC. However, a positive association was observed for sRAGE. Our findings may stimulate further research on the role of AGEs and sRAGE in survival among cancer patients with special emphasis on potential effect modifications by sex and diabetes.

Abstract Word count: 248

Word count: 4,076

Number of Tables & Figures: 3 tables & 2 figures

References: 40

INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer worldwide, and the second leading cause of death by cancers, accounting for over 9% of all cancer deaths.[1] The number of CRC survivors is increasing, and thus 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) cancer prevention recommendations are potentially associated with poorer survival among individuals with CRC.[3, 4] Furthermore, hyperglycemia, diabetes, and insulin resistance have been studied and shown to be associated with lower survival among patients with CRC.[5-7]

Advanced glycation end-products (AGEs) are a heterogeneous group of molecules formed by a nonenzymatic glycation reaction, also known as the Maillard reaction.[8, 9] Circulating AGEs including N^{ε}-[carboxy-methyl]lysine (CML), N^{ε}-[carboxy-ethyl]lysine (CEL) and N^{δ}-[5-hydro-5-methyl-4-imidazolon-2-yl]-ornithine (MG-H1), may serve as a proxy of unhealthy dietary choices. [10] Foods rich in both fat and protein, and cooked at high temperature and with dry heat processing tend to be the major sources of dietary AGEs.[8] Endogenous formation of AGEs is a normal by-product of metabolic processes but occurs at faster rates in the presence of hyperglycemia, a common state in diabetes.[8] Both exogenous and endogenous AGEs are similar in their biological functions and were shown to promote oxidative stress, inflammation, and cellular damage, contributing to the development and progression of multiple diseases.[9]

AGEs elicit biological function by binding to their receptor (RAGE), which has a low expression in human tissues, but was found to be overexpressed in tumors of the colon, breast, brain, prostate, and ovary.[11-13] Higher amounts of AGEs were also observed in tumor tissues.[9] The binding of AGEs to RAGE activates extracellular matrix glycation, NADPH oxidase activity, VEGF expression, local hypoxia, and NF-κB to promote oxidative stress and inflammation, which could enhance tumor angiogenesis and proliferation.[9, 14] Unlike RAGE, binding of AGEs to the soluble receptor for AGEs (sRAGE), a truncated form of RAGE without the trans-membrane domain, does not trigger inflammation, and circulating sRAGE has been associated with a lower risk of CRC in observational studies.[15-17] Despite promising evidence from experimental studies, there were no human studies on AGEs and sRAGE among CRC survivors.

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 in a large, multicenter prospective cohort study,

the European Prospective Investigation into Cancer and Nutrition (EPIC). We also studied various ratios of AGEs to better understand the chemical origin of individual AGEs and their association with mortality among CRC patients.[18]

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 located 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 previously published.[19] 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 cancer onset or diagnosis. Diet over the previous 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 collected, and isolated plasma 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) and Sweden (-80^{o}C at Malmö and Umeå). Individuals who were eligible for EPIC were selected from the general population of a specific geographical area, town, or province. Exceptions included the French sub-cohort, which was based on members of the national health insurance system or state-school employees, and the Utrecht (Netherlands) sub-cohort, which was based on women who underwent breast cancer screening. The present analysis is based on participant data from all centers except for Greece (excluded due to data restriction issues). Methods for follow-up of cancer incidence were previously described.[19]

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 December 2009 and March 2014 for Italy, March 2010 and January 2015 for Germany, December 2012 and December 2013 for Spain, December 2012 for the Netherlands and Norway, July 2013 for Denmark, October 2013 for France, December 2013 for the United Kingdom, and February 2014 for Sweden. 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.

Case ascertainment and selection

Cancer data were coded using the ICD-10 and the second revision of the International Classification of Disease for Oncology (ICD-O-2). CRC cases were selected from participants who developed colon (C18.0-C18.7), rectal (C19-C20), and overlapping or unspecified origin tumors (C18.8-C18.9). Anal cancers (C21) were excluded. 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, and five non-adenocarcinoma cases and one case with a very high implausible value (>mean+2 SD) of CML were also excluded, resulting in 1,369 CRC cases for AGEs analyses. Additionally, in sRAGE analysis, 23 cases were excluded due to missing sRAGE measurements resulting in 1,346 CRC cases.

Biomarker Measurements

Plasma concentrations of protein-bound CML, CEL and MG-H1 were measured in the laboratory of Prof. Schalkwijk (Maastrich University, Netherlands) using ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) as previously described.[20 21] In brief, CML, CEL and MG-H1 were extracted from plasma using butanolic hydrochloric acid and analyzed in positive electrospray ionization multiple reaction monitoring mode. AGEs were quantified by calculating the area ratio of each unlabeled peak area to the corresponding internal standard. The sum of AGEs (ΣAGEs, in nmol/L) was calculated by summing up the concentrations of CML, CEL and MG-H1 for each subject. We further calculated the ratios of the AGEs considering their dicarbonyl intermediates: methylglyoxal (MGO)-derived/

glyoxal (GO) derived (i.e. CEL+MG-H1 divided by CML) (**Supplementry Figure 1**).[21] 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).

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 [22]. Intra- and inter-batch coefficients of variation (CVs) were assessed by measuring three different samples used as quality controls in duplicate in each batch. Mean intra- and inter-batch CVs for sRAGE were 1.25% and 6.0%, respectively. [15]

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, and were expressed in U.S. National Glycohemoglobin Standardization Program units and as percentages of hemoglobin.[23] A total of 1,026 incident CRC cases (561 men and 465 women) had a prediagnostic HbA1c measurement.

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 (continuous) and age at death or censor (continuous) were used as the start and end times for follow-up. Correlations between AGEs and sRAGE were assessed using Spearman's rank correlation coefficients in all participants (**Supplementary Figure 2**).

Cox proportional hazards (PH) models stratified by center and adjusted for age at diagnosis (continuous), sex (men, women), and tumor stage (I, II, III, IV, missing) were used to calculate hazard ratios (HRs) and 95% confidence intervals (CIs) for the association between CML, CEL, MG-H1, sRAGE, ΣAGEs, and selected ratios (CEL/MG-H1 and [CEL+MG-H1]/CML) and CRC-specific and overall mortality. Each biomarker or ratio of interest was examined in quintiles and as a continuous variable per one standard deviation (SD) increase of the respective biomarker or ratio.

The quintile cut-points were defined based on the distribution of biomarkers among all CRC cases. *P*-values for linear trend were calculated with the median value of each quintile included as a continuous variable in the corresponding model.

To determine the final model, the following *a priori* identified covariates were assessed as potential confounders by evaluating if there was a sizeable change in HRs after including them in the model: grade of tumor differentiation (well, moderately, poorly differentiated, missing), location of primary tumor (colon, rectum), smoking status (never, former, current, missing), body mass index (BMI) (kg/m^2), year of diagnosis (continuous), self-reported diabetes status at baseline or identified between baseline and cancer diagnosis (yes, no, missing), daily dietary intakes of total energy (continuous), red and processed meats (continuous), fiber (continuous), dairy (continuous), alcohol (continuous) and total sugars (continuous). These variables were chosen based on previous published evidence showing their associations with CRC incidence or survival and/or blood AGEs or sRAGE concentrations. While adjusting for these variables had no substantial influence on the effect estimates, we chose to additionally control for the following variables in the final model to account for factors potentially associated with CRC survival: location of primary tumor (colon, rectum), smoking status (never, former, current, missing), BMI (kg/m^2; continuous), year of diagnosis (continuous), and diabetes (yes, no, missing).

The PH assumption was assessed graphically by estimating "log-log" survival curves. If the curves were approximately parallel, the PH assumption was deemed reasonable. In addition, the PH assumption was verified using goodness of fit test methods and checking correlations between Schoenfeld residuals and time dependent variables in the Cox model.

Information regarding categorization and harmonization of tumor stage data has been previously published.[24] 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.[25] 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. The sensitivity analysis by fasting status indicated no differences in the results by timing of blood draw. Additional sensitivity analyses were performed by length of follow-up after cancer diagnosis (≥2, ≥3, and ≥5 years) and time between blood collection and cancer diagnosis (tertiles, <2.9, 2.9-5.59, and ≥5.6 years) to assess whether the associations differ by length of survival and timing of biomarker measurements, respectively. Finally, thirty study participants with unknown cause of death were included only in all-cause mortality analyses as non-CRC deaths. A sensitivity analysis showed that the CRC-specific and all-cause mortality results are not affected by how cause of death is coded for these thirty cases (due to CRC or non-CRC causes).

We assessed the linearity of the association between each biomarker and risk for CRC-specific or all-cause mortality using non-parametric restricted cubic splines fitted to a Cox PH model using the SAS macro "lgtphcurv9". The likelihood ratio test comparing the model with only the linear term to the model with the linear and cubic spline terms was used to calculate *P*-values. All models were consistent with a linear response (**Supplementary Figures 3-6**).

Subgroup analyses by categories of potentially biologically relevant effect modifiers (sex [men *vs*. women], age at diagnosis [< *vs*. ≥ median], tumor anatomical site [colon *vs*. rectum], colon anatomical subsite [proximal *vs.* distal], tumor stage [I-II *vs.* III-IV], year of diagnosis [< *vs*. ≥ median], BMI [<25, 25-29.9 *vs*. ≥30], physical activity [inactive, moderately inactive, moderately active, active], smoking status [never, former, current], diabetes [yes *vs*. no], and categories of circulating HbA1c [normal < 42 *vs*. pre-diabetes/diabetes ≥ 42 mmol/mol]) were conducted for 1 SD increase in each biomarker. 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. 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 9.4 (SAS Institute, Cary, NC, USA) and R version 4.3.1 and *P*-values of < *0.05* were considered statistically significant.

RESULTS

Baseline characteristics of study participants

The distribution of selected baseline characteristics of CRC cases according to quintiles of all AGEs (CML+CEL+MG-H1) are shown in **Table 1** and according to quintiles of individual biomarkers (CML, CEL, MG-H1, and sRAGE) in **Supplementary Tables 1-4**. Among 1,369 eligible CRC cases, there were 693 deaths (including 541 deaths from CRC, 62 from other malignant neoplasms, 60 from non-cancer causes, and 30 with unknown causes of death). Median follow up time was 103 months (interquartile range, IQR: 23- 155 months). CML, CEL, MG-H1, and sRAGE measurements were on average 53 (SD=33) months before CRC diagnosis.

Individual AGEs, sRAGE 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**. None of the individual AGE biomarkers were statistically significantly associated with either CRC-specific or overall mortality. However, a potential effect modification by sex was observed for CEL and CRC-specific mortality, with the multivariable-adjusted HR for one SD increase in CEL concentration being 1.19 (95% CI: 1.01-1.40) for women and 0.82 (95% CI: 0.66-1.03) for men ($P_{\text{interaction by sex}} = 0.05$). High sRAGE concentrations were positively associated with CRC-specific ($HR_{Q5\ vs\ Q1} = 1.67$, 95% CI: 1.21-2.30, $P_{trend}=0.02$) and all-cause mortality ($HR_{Q5\ vs\ Q1} = 1.38$, 95% CI: 1.05-1.83, $P_{trend} = 0.09$).

Combined AGEs, AGE ratios and mortality among CRC patients

We also analyzed the combined AGEs as a marker of cumulative exposure to AGEs and several ratios to assess different AGE pathways. Since CEL and MG-H1 both have common pathways that derive from methylglyoxal, we examined the ratio of CEL/MG-H1 and (CEL+MG-H1)/CML. For the combined AGEs and the ratios CEL/MG-H1 and (CEL+MG-H1)/CML, the associations with CRC-specific mortality were largely null: (CML+CEL+MG-H1), $HR_{Q5\ vs\ Q1}$ = 0.98, 95% CI: 0.70-1.37, P_{trend} = *0.89;* CEL/MG-H1: $HR_{Q5\ vs\ Q1}$ = 0.91, 95% CI: 0.63-1.31, P_{trend} = *0.95*; (CEL+MG-H1)/CML: $HR_{Q5\ vs\ Q1}$ = 1.06, 95% CI: 0.69-1.62, P_{trend} = *0.24* **(Table 3)**. Similar results were observed for all-cause mortality (Table 3).

Stratified analyses

Subgroup analyses showed differences in the associations between CML, CEL, MG-H1, sRAGE and CRC-specific mortality across select subcategories of potential *a priori* defined biologically plausible effect modifiers (**Figure 1 and Supplementary Table 5**). The results for all-cause mortality are shown in **Supplementary Table 6**. The associations of individual AGEs and sRAGE with CRC-specific mortality were suggestively stronger and positive among CRC cases with diabetes. In a joint analysis of sRAGE and HbA1c concentrations, CRC cases in the highest sRAGE tertile (≥1,191.65 ng/ml) with HbA1c concentrations consistent with pre-diabetes and diabetes (≥42 mmol/mol) were at the highest risk of dying from CRC (HR = 2.05, 95% CI: 1.35-3.13; $P_{interaction}$ <*0.01*) and all-cause mortality (HR = 1.93, 95% CI: 1.33-2.81; $P_{interaction}$ = *0.01*) compared to cases in the lowest sRAGE tertile with normal HbA1c concentrations (<42 mmol/mol; **Figure 2 and Supplementary Figure 7**).

Sensitivity Analyses

For all biomarkers, similar results were obtained in complete CRC stage and imputed CRC stage data analyses for CRC-specific and all-cause mortality (**Figure 1** and **Supplementary Tables 5-6**). After exclusion of cases who died during the first two years of follow-up after cancer diagnosis, the overall findings did not change substantially. Additionally, in a stratified analysis by time between blood collection and CRC diagnosis, the AGEs seemed to be most strongly positively associated with CRC-specific mortality if measured more than 5.6 years before cancer diagnosis (HR = 1.30, 95% CI: 1.04-1.63; $P_{interaction}$ < *0.01*; **Figure 1**).

DISCUSSION

This study is the first prospective analysis of the association of pre-diagnostic circulating AGEs and sRAGE with mortality among CRC patients. The results of this study showed that plasma concentrations of AGEs were not associated with CRC-specific and overall mortality. However, we observed a positive association between circulating sRAGE and CRC-specific and all-cause mortality. Our findings also suggested that the results for individual AGEs and sRAGE may differ by diabetes status (self-reported and as defined by HbA1c concentrations).

We originally hypothesized that AGEs are positively associated with CRC-specific and all-cause mortality among individuals with CRC due to AGEs' adverse effects on cell function.[18] AGEs bind to cell-bound receptor for AGEs resulting in generation of reactive oxygen species and increased production of pro-inflammatory factors.[18] Furthermore, RAGE activation and overexpression have been shown to play a role in inflammation-associated colon carcinogenesis and to increase colon tumor cell migration and proliferation,[26, 27] while inhibiting expression of RAGE suppressed colon cancer cell angiogenesis.[28] Despite biologic plausibility, there were no epidemiologic studies that investigated the association between circulating AGEs and survival among CRC patients. An observational study among 1,018 breast cancer patients from China found that compared with the first quartile of blood AGEs measured at diagnosis, the fourth quartile was associated with higher all-cause mortality (HR = 1.86, 95% CI: 1.03-3.36).[29] Another study was conducted among 2,039 postmenopausal women diagnosed with breast cancer in the Women's Health Initiative (WHI) study. It found that higher dietary CML intake after breast cancer diagnosis was associated with higher risk of all-cause (HR = 1.51, 95% CI: 1.17-1.94), cardiovascular disease (CVD, HR = 2.14, 95% CI: 1.19-3.84), and breast cancer mortality (HR = 1.86, 95% CI: 1.19-2.91).[30] <="" span="">*vs*. after cancer diagnosis), AGE assessment methods (blood *vs*. diet; multiple AGEs *vs*. only CML), study populations (CRC *vs*. breast cancer survivors; men and women *vs.* only women), our results among patients with CRC may not be directly comparable with these findings. We have previously published results from EPIC on the association between pre-diagnostic dietary intakes of three AGEs (CML, CEL, and MG-H1) and CRC-specific and all-cause mortality among individuals diagnosed with CRC. Consistent with our results

for circulating AGEs described here, we found no statistically significant associations between pre-diagnostic dietary AGEs and CRC-specific or all-cause mortality among individuals with CRC.[31] An exception could be our finding for circulating CEL for which there were suggestive positive associations with CRC-specific and all-cause mortality in women and inverse associations in men. Therefore, it is possible that the associations between AGEs and mortality among cancer survivors differ by sex, and that sex hormones, in part, are responsible for this.[32, 33] Also, the AGEs seemed to be most strongly positively associated with mortality if measured more than 5.6 years before cancer diagnosis. While the importance of this finding is not clear, it may suggest that the AGEs could influence tumor aggressiveness. More research is needed to further explore these observations.

We hypothesized that circulating sRAGE is inversely associated with CRC-specific and all-cause mortality among individuals with CRC as sRAGE is a soluble decoy receptor for AGEs and does not produce the same inflammatory effects as AGEs binding with RAGE.[11] Additionally, sRAGE has been shown to contribute to anti-atherosclerosis effects through oxidized low-density lipoprotein (ox-LDL) quenching. Ox-LDL is produced in excess in CRC tissue and is part of several mechanisms closely linked to tumorigenesis.[34] Furthermore, evidence from observational studies on CRC risk, including ours in EPIC,[15] supported an inverse association between sRAGE and CRC development.[15, 17, 35] Our previously conducted nested case-control study in EPIC found that sRAGE concentrations were inversely associated with CRC risk ($RR_{Q5\ vs.Q1}$= 0.77, 95% CI: 0.59-1.00) in all participants and, in particular, among men ($RR_{Q5\ vs.Q1}$ = 0.63, 95% CI: 0.42-0.94).[17] Another prospective case-cohort study among Finnish male smokers suggested an inverse association of serum sRAGE with risk of CRC ($RR_{Q5\ vs.Q1}$ = 0.65; 95% CI: 0.39-1.07; P_{trend} = *0.03*).[17] In the WHI study, blood sRAGE concentrations were inversely associated with risk of CRC among post-menopausal women with BMI > 25 kg/m^2 ($HR_{Q4\ vs.Q1}$ = 0.39, 95% CI: 0.17-0.91), but not among women with BMI <25 kg/m^2.[16, 35] There were no previous studies of sRAGE among CRC survivors. A prospective cohort study among melanoma cancer patients (n = 229) found that lower serum sRAGE concentrations at diagnosis were associated with higher mortality among stage III/IV patients ($HR_{lower\ vs.\ higher}$ = 1.9, 95% CI: 1.2-3.1).[36] It was recently proposed that a high concentration of sRAGE may be an indicator of chronic inflammation and multimorbidity rather than a healthy

state,[37] which can in part explain our finding of a positive association between sRAGE and mortality in our population. However, more research is needed to validate this finding.

In our subgroup analysis, sRAGE was strongly positively associated with CRC-specific mortality among individuals with diabetes. We previously observed a similar effect modification by diabetes status between dietary AGEs and CRC-specific and overall mortality in the same population.[31] AGEs production is enhanced under hyperglycemic conditions. 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.[14, 38] Glucose-derived AGEs can bind to RAGE and upregulate the expression of carbohydrate-responsive element-binding protein (ChREBP) under glucose-free conditions to promote the proliferation of CRC cells.[39] The study of Deng *et al.* showed that AGEs promoted invasion and migration of CRC partially through the RAGE/ERK/Specificity Protein 1 (Sp1)/MMP2 cascade, possibly supporting the reason for the poor prognosis of CRC in diabetic patients.[40] Additionally, poor kidney function, which is more common in diabetic individuals, is associated with AGE accumulation. In our study, the comparison of participants based on joint consideration of sRAGE and HbA1c showed that individuals in the highest sRAGE tertile and who had HbA1c classified as pre-diabetic /diabetic had the highest mortality rates, possibly, due to poor diets. Once patients are diagnosed as pre- or diabetic, significant dietary adjustments are recommended, which could help lower exogenous AGEs intake.

The strengths of this study included its design as a large prospective study which allowed for stratification by sex and other biologically plausible effect modifiers. Additionally, we were able to control for multiple confounders. Accounting for missing information on CRC stage through sensitivity analysis and imputation clarified the impact of missing stage data on the results. Finally, the measurement of AGEs through biomarkers rather than dietary questionnaires strengthened the accuracy of the AGEs' measurements.

Our study also had several limitations. First, our population was conducted in Western Europeans and therefore the results might not be generalizable to CRC survivors from other populations. However, our study included a relatively wide range of ages and both men and women.

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 and progression. Finally, we were unable to test colon or rectal tumor tissue samples for AGEs and RAGE. It is plausible that colorectal tumors may produce AGEs, which can be attributed at least in part to the metabolic changes that happen in cancer cells as Warburg effect of cancer cells increase glycolysis leading to accumulation of AGEs precursors. [41]

The results of this large observational study in Western European populations suggest that plasma concentrations of AGEs are not associated with CRC-specific and overall mortality. However, pre-diagnostic concentrations of sRAGE were positively associated with CRC-specific and overall mortality. Potential effect modification by HbA1c, diabetes, sex, and time between blood collection and follow-up may impact these associations. 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.

Acknowledgments and Funding

Grant support: Funding [WCRF 2015/1391, PI: M. Jenab] was obtained from Wereld Kanker Onderzoek Fonds (WKOF), as part of the World Cancer Research Fund International grant programme.

The coordination of EPIC is financially supported by International Agency for Research on Cancer (IARC) and also by the Department of Epidemiology and Biostatistics, School of Public Health, Imperial College London which has additional infrastructure support provided by the NIHR Imperial Biomedical Research Centre (BRC). The national cohorts are supported by: Danish Cancer Society (Denmark); Ligue Contre le Cancer, Institut Gustave Roussy, Mutuelle Générale de l'Education Nationale, Institut National de la Santé et de la Recherche Médicale (INSERM) (France); German Cancer Aid, German Cancer Research Center (DKFZ), German Institute of Human Nutrition PotsdamRehbruecke (DIfE), Federal Ministry of Education and Research (BMBF) (Germany); Associazione Italiana per la Ricerca sul Cancro-AIRC-Italy, Compagnia di SanPaolo and National Research Concil (Italy); Dutch Ministry of Public Health, Welfare and Sports (VWS), Netherlands Cancer Registry (NKR), LK Research Funds, Dutch

Prevention Funds, Dutch ZON (Zorg Onderzoek Nederland), World Cancer Research Fund (WCRF), Statistics Netherlands (The Netherlands); Health Research Fund (FIS) - Instituto de Salud Carlos III (ISCIII), Regional Governments of Andalucía, Asturias, Basque Country, Murcia and Navarra, and the Catalan Institute of Oncology - ICO (Spain); Swedish Cancer Society, Swedish Research Council and County Councils of Skåne and Västerbotten (Sweden); Cancer Research UK (14136 to EPIC-Norfolk; C8221/A29017 to EPIC-Oxford), Medical Research Council (1000143 to EPIC-Norfolk; MR/M012190/1 to EPIC-Oxford; United Kingdom).

V. Fedirko is supported by the Cancer Prevention and Research Institute of Texas (CPRIT) Rising Stars Award (Grant ID RR200056). DJ. Hughes is supported by the Health Research Board of Ireland health research award HRA-POR-2013-397. DJ. Hughes and M. Jenab are supported by the EU Marie Curie Doctoral Training Network 'Colomark' (https://www.colomark.org/).

The EPIC-Norfolk study (DOI 10.22025/2019.10.105.00004) has received funding from the Medical Research Council (MR/N003284/1 and MC-UU_12015/1) and Cancer Research UK (C864/A14136). We are grateful to all the participants who have been part of the project and to the many members of the study teams at the University of Cambridge who have enabled this research. We thank the National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands, for their contribution and ongoing support to the EPIC Study. We acknowledge the use of data from the EPIC-Aarhus cohort, EPIC-Asturias cohort, and EPIC-Swedish cohort. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Data Availability Statement

The data that support the findings of this study are available from the corresponding authors upon reasonable request. Data access is regulated by the EPIC rules and requires the EPIC Steering Committee approval. For information on how to submit an application for gaining access to EPIC data and/or biospecimens, please follow the instructions at http://epic.iarc.fr/access/index.php.

Ethics Statement

The EPIC study was approved by the Ethical Review Board of the IARC and the Institutional Review Boards of each participating center. Written consent was obtained from all EPIC participants at enrollment into the study.

Disclaimer

Where authors are identified as personnel of the International Agency for Research on Cancer/World Health Organization, the authors alone are responsible for the views expressed in this article and they do not necessarily represent the decisions, policy or views of the International Agency for Research on Cancer / World Health Organization. The funding sources had no influence on the design of the study; the collection, analysis, and interpretation of data; the writing of the report; or the decision to submit the paper for publication.

Author Contributions

Conceptualization, M.J. and V.F.; Data curation, J.L., J.R., M.J., and V.F.; Formal analysis, J.L., Z.Z. and J.R.; Funding acquisition, M.J. and V.F.; Investigation, M.J., C.S., and all EPIC co-authors (A.K.E., A.T., G.S., V.K., R.K., M.B.S., G.M., V.P., F.P., R.T., L.P., R.C.H.V., I.T.G., T.B., P.G.J., M-J.S., J-H.G-G., C.M-I., P.A., K.P., E.W., A.K.H.); Methodology, M.J. and V.F.; Project administration, M.J. and V.F.; Resources, M.J., V.F., and all EPIC co-authors (A.K.E., A.T., G.S., V.K., R.K., M.B.S., G.M., V.P., F.P., R.T., L.P., R.C.H.V., I.T.G., T.B., P.G.J., M-J.S., J-H.G-G., C.M-I., P.A., K.P., E.W., A.K.H.); Supervision, M.J. and V.F.; Writing—original draft, J.L., J.R., E.K.A., M.J., and V.F.; Writing—review and editing, J.L., E.K.A., M.J., L.J., H.F., D.J.H., C.S., M.J., V.F. and all EPIC co-authors (A.K.E., A.T., G.S., V.K., R.K., M.B.S., G.M., V.P., F.P., R.T., L.P., R.C.H.V., I.T.G., T.B., P.G.J., M-J.S., J-H.G-G., C.M-I., P.A., K.P., E.W., A.K.H.). The work reported in the paper has been performed by the authors, unless clearly specified in the text.

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Table 1. Selected baseline characteristics of CRC cases (N = 1,369) according to quintiles of the sum of circulating AGEs (ΣAGEs = CML+CEL+MG-H1, nmol/L) in the EPIC study.

Baseline characteristic	All AGEs (CML+CEL+MG-H1), nmol/L				
	Quintile 1: ≤ 4176 N=273	**Quintile 2: 4177-4664 N = 274**	**Quintile 3: 4665-5168 N = 274**	**Quintile 4: 5169-6031 N = 274**	**Quintile 5: ≥ 6032 N = 274**
All AGEs, mean (SD), nmol/L	3755.4 (353.8)	4423.2 (141.0)	4912.9 (148.3)	5531.0 (237.3)	7542.9 (1506.8)
Age at diagnosis, mean (SD), yr	61.8 (7.5)	62.7 (8.1)	62.0 (7.5)	63.1 (7.6)	63.4 (7.5)
Men, N (%)	137 (50)	146 (53)	134 (49)	125 (46)	122 (45)
Tumor stage, N (%)[a]					
I	68 (25)	66 (24)	93 (34)	66 (24)	60 (22)
II	68 (25)	53 (19)	52 (19)	52 (19)	46 (17)
III	79 (29)	88 (32)	78 (28)	79 (29)	86 (31)
IV	37 (14)	34 (12)	22 (8)	31 (11)	33 (12)
Location of primary tumor, N (%)					
Colon[b]	187 (68)	179 (65)	183 (67)	162 (59)	159 (58)
Rectum	86 (32)	95 (35)	91 (33)	112 (41)	115 (42)
Yr at diagnosis, mean (min-max)	1999 (1993-2010)	1999 (1993-2010)	2000 (1993-2012)	2000 (1994-2009)	2000 (1993-2010)
Smoking status, N (%)[a]					
Never	103 (38)	112 (41)	119 (43)	126 (46)	107 (39)
Former	78 (29)	91 (33)	92 (34)	97 (35)	105 (38)
Current	87 (32)	69 (25)	61 (22)	48 (18)	61 (22)
BMI, mean (SD), kg/m^2	28.3 (4.9)	27.1 (4.1)	26.2 (4.0)	26.2 (4.0)	25.7 (4.0)
Physical activity, N (%)[a,c]					
Inactive	50 (18)	53 (19)	41 (15)	36 (13)	45 (16)
Moderately inactive	84 (31)	70 (26)	64 (23)	76 (28)	91 (33)
Moderately active	108 (40)	117 (43)	106 (39)	118 (43)	97 (35)
Active	27 (10)	17 (6)	30 (11)	23 (8)	30 (11)
Diabetes, N (%)[a,d]	17 (6)	13 (5)	10 (4)	12 (4)	9 (3)
Dietary fiber, mean (SD), g[e]	21.6 (7.6)	23.0 (7.9)	22.5 (7.9)	22.9 (7.4)	24.3 (8.3)
Dietary calcium, mean (SD), mg[e]	927.2 (414.5)	1016.48 (437.4)	974.7 (387.3)	1024.5 (447.0)	1028.2 (419.7)
Vegetables & fruits, mean (SD), g[e]	405.9 (257.2)	403.76 (226.6)	384.0 (217.0)	385.9 (224.4)	411.7 (253.4)

Red & processed meat, mean (SD), g[e]	92.8 (54.3)	89.02 (51.2)	90.8 (101.0)	83.4 (48.2)	86.5 (55.9)
Energy intake, mean (SD), kcal[e]	2117 (618)	2228 (745)	2122 (870)	2131 (682)	2135 (652)

Abbreviations: AGES, advanced glycation end products; SD, standard deviation; N, Number of participants; yr, year, min, minimum; max, maximum; kcal, kilocalories; kg, kilograms; m, meters; mg, milligram; g, gram; d, days.

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

[b] Colon cancer includes cancers in the proximal, distal and unidentified colon site.

[c] Sex-specific categories.

[d] This category includes self-reported cases of diabetes at baseline and new incident cases identified between baseline and cancer diagnosis.

[e] Daily dietary intakes. Missing values were imputed with sex-specific dietary medians for: dietary fiber (n = 3), dietary calcium (n = 3), vegetables & fruits (n = 3), red & processed meat (n = 3), energy intake (n = 3).

Table 2. Multivariable-adjusted HRs and 95% CIs for CRC-specific and all-cause mortality according to quintiles of individual circulating AGEs (CML, CEL, MG-H1) and sRAGE in the EPIC study.

Biomarker/ Outcome/ Category	Cut-offs[a]	**Combined** Deaths/ Total	**Combined** HR (95% CI)[b,c]	**Men** Deaths/ Total	**Men** HR (95% CI)[b,c]	**Women** Deaths/ Total	**Women** HR (95% CI)[b,c]	$P_{interaction}$[c,d,e]
CML, nmol/L								
CRC-specific mortality								
Quintile 1	≤ 1975	107/272	1.00 (ref)	58/146	1.00 (ref)	49/126	1.00 (ref)	
Quintile 2	[1976-2286]	110/268	0.95 (0.69-1.31)	46/116	0.77 (0.48-1.24)	64/152	1.09 (0.67-1.76)	
Quintile 3	[2287-2648]	103/267	0.98 (0.71-1.36)	55/126	1.14 (0.69-1.87)	48/141	0.83 (0.51-1.37)	
Quintile 4	[2649-3218]	104/268	0.90 (0.64-1.27)	52/125	1.08 (0.63-1.85)	52/143	0.69 (0.42-1.16)	
Quintile 5	≥ 3219	117/264	0.80 (0.55-1.17)	60/133	0.89 (0.51-1.55)	57/131	0.73 (0.41-1.32)	*0.20*
P_{trend}[f]			*0.24*		*0.85*		*0.11*	
Per 1 SD[g]		541/1339	1.01 (0.90-1.13)	271/646	1.02 (0.87-1.2)	270/693	1.00 (0.82-1.2)	*0.77*
All-cause mortality								
Quintile 1	≤ 1975	138/273	1.00 (ref)	78/146	1.00 (ref)	60/127	1.00 (ref)	
Quintile 2	[1976-2286]	145/274	0.99 (0.75-1.31)	63/120	0.81 (0.54-1.22)	82/154	1.26 (0.82-1.93)	
Quintile 3	[2287-2648]	131/274	0.91 (0.68-1.21)	74/131	0.99 (0.64-1.51)	57/143	0.81 (0.52-1.27)	
Quintile 4	[2649-3218]	131/274	0.84 (0.62-1.13)	63/128	0.80 (0.51-1.28)	68/146	0.83 (0.53-1.31)	
Quintile 5	≥ 3219	148/274	0.73 (0.53-1.02)	80/139	0.73 (0.45-1.18)	68/135	0.85 (0.51-1.42)	*0.16*
P_{trend}[f]			*0.10*		*0.52*		*0.11*	
Per 1 SD[g]		693/1369	0.97 (0.87-1.08)	358/664	0.97 (0.84-1.13)	335/705	1.02 (0.86-1.22)	*0.83*
CEL, nmol/l								
CRC-specific mortality								
Quintile 1	≤ 981	114/266	1.00 (ref)	68/140	1.00 (ref)	46/126	1.00 (ref)	
Quintile 2	[982-1241]	102/271	0.75 (0.55-1.02)	55/136	0.60 (0.40-0.91)	47/135	1.06 (0.63-1.77)	

Quintile 3	[1242-1476]	104/267	0.89 (0.65-1.22)	53/130	0.74 (0.48-1.15)	51/137	1.12 (0.68-1.85)	
Quintile 4	[1477-1785]	110/266	0.96 (0.71-1.31)	46/117	0.66 (0.43-1.02)	64/149	1.27 (0.77-2.09)	
Quintile 5	≥ 1786	111/269	0.96 (0.69-1.35)	49/123	0.66 (0.41-1.08)	62/146	1.37 (0.77-2.44)	*0.28*
P_{trend}[f]			*0.40*		*0.19*		*0.06*	
Per 1 SD[g]		541/1339	1.02 (0.91-1.15)	271/646	0.82 (0.66-1.03)	270/693	1.19 (1.01-1.40)	*0.05*
All-cause mortality								
Quintile 1	≤ 981	148/273	1.00 (ref)	89/144	1.00 (ref)	59/129	1.00 (ref)	
Quintile 2	[982-1241]	137/274	0.72 (0.55-0.94)	73/137	0.58 (0.40-0.83)	64/137	1.02 (0.65-1.61)	
Quintile 3	[1242-1476]	134/274	0.80 (0.61-1.05)	74/136	0.66 (0.46-0.96)	60/138	1.03 (0.66-1.62)	
Quintile 4	[1477-1785]	141/274	0.89 (0.68-1.16)	61/121	0.66 (0.45-0.96)	80/153	1.20 (0.77-1.87)	
Quintile 5	≥ 1786	133/274	0.85 (0.63-1.14)	61/126	0.63 (0.41-0.95)	72/148	1.23 (0.74-2.06)	*0.15*
P_{trend}[f]			*0.89*		*0.04*		*0.12*	
Per 1 SD[g]		693/1369	0.96 (0.86-1.07)	358/664	0.78 (0.65-0.95)	335/705	1.14 (0.98-1.32)	*0.07*
MG-H1, nmol/l								
CRC-specific mortality								
Quintile 1	≤ 857	107/270	1.00 (ref)	61/143	1.00 (ref)	46/127	1.00 (ref)	
Quintile 2	[858-953]	106/268	1.00 (0.74-1.37)	56/132	1.08 (0.70-1.66)	50/136	0.90 (0.55-1.47)	
Quintile 3	[954-1058]	108/266	1.07 (0.78-1.46)	48/117	1.03 (0.64-1.64)	60/149	0.91 (0.56-1.49)	
Quintile 4	[1059-1221]	101/271	0.91 (0.66-1.26)	47/124	0.86 (0.54-1.38)	54/147	0.85 (0.52-1.40)	
Quintile 5	≥ 1222	119/264	1.02 (0.75-1.40)	59/130	0.90 (0.58-1.41)	60/134	1.05 (0.64-1.75)	*0.83*
P_{trend}[f]			*0.50*		*0.98*		*0.62*	
Per 1 SD[g]		541/1339	1.06 (0.96-1.17)	271/646	0.99 (0.86-1.14)	270/693	1.15 (0.98-1.35)	*0.31*
All-cause mortality								
Quintile 1	≤ 857	136/273	1.00 (ref)	79/145	1.00 (ref)	57/128	1.00 (ref)	
Quintile 2	[858-953]	139/274	1.00 (0.76-1.31)	72/134	1.02 (0.70-1.48)	67/140	0.96 (0.62-1.48)	
Quintile 3	[954-1058]	143/274	1.03 (0.79-1.36)	69/123	1.02 (0.69-1.51)	74/151	0.95 (0.61-1.47)	
Quintile 4	[1059-1221]	126/274	0.84 (0.63-1.12)	61/126	0.80 (0.54-1.20)	65/148	0.78 (0.50-1.22)	
Quintile 5	≥ 1222	149/274	0.97 (0.74-1.29)	77/136	0.88 (0.60-1.30)	72/138	1.04 (0.66-1.64)	*0.91*
P_{trend}[f]			*0.72*		*0.99*		*0.76*	
Per 1 SD[g]		693/1369	1.02 (0.93-1.11)	358/664	0.97 (0.86-1.10)	335/705	1.09 (0.95-1.26)	*0.38*
sRAGE, ng/ml[h]								
CRC-specific mortality								
Quintile 1	≤ 711	100/261	1.00 (ref)	64/168	1.00 (ref)	36/93	1.00 (ref)	
Quintile 2	[712-903]	111/264	1.10 (0.81-1.49)	70/153	1.12 (0.76-1.67)	41/111	0.93 (0.54-1.59)	
Quintile 3	[904-1103]	101/263	1.11 (0.81-1.52)	46/126	0.89 (0.57-1.38)	55/137	1.00 (0.59-1.69)	
Quintile 4	[1104-1403]	99/264	1.16 (0.84-1.60)	33/89	1.01 (0.61-1.67)	66/175	1.06 (0.64-1.75)	
Quintile 5	≥ 1404	116/264	1.67 (1.21-2.30)	44/88	1.70 (1.06-2.74)	72/176	1.46 (0.89-2.40)	*0.84*
P_{trend}[f]			*0.02*		*0.14*		*0.20*	
Per 1 SD[g]		527/1316	1.21 (1.10-1.33)	257/624	1.22 (1.05-1.41)	270/692	1.18 (1.02-1.37)	*0.32*

All-cause mortality								
Quintile 1	≤ 711	132/269	1.00 (ref)	85/173	1.00 (ref)	47/96	1.00 (ref)	
Quintile 2	[712-903]	153/269	1.14 (0.88-1.48)	96/157	1.09 (0.78-1.52)	57/112	1.09 (0.69-1.72)	
Quintile 3	[904-1104]	131/270	1.12 (0.85-1.47)	61/129	0.93 (0.64-1.35)	70/141	1.01 (0.64-1.61)	
Quintile 4	[1105-1403]	124/269	1.13 (0.86-1.50)	46/92	1.04 (0.69-1.58)	78/177	1.10 (0.71-1.71)	
Quintile 5	≥ 1404	137/269	1.38 (1.05-1.83)	54/91	1.33 (0.89-2.00)	83/178	1.40 (0.90-2.19)	*1.00*
P_{trend}[f]			*0.09*		*0.16*		*0.42*	
Per 1 SD[g]		677/1346	1.14 (1.04-1.25)	342/642	1.16 (1.02-1.33)	335/704	1.15 (1.00-1.31)	*0.36*

Abbreviations: AGES, advanced glycation end products; CML, Nε-(carboxy-methyl)lysine; CEL, Nε-(carboxy-ethyl)lysine; MG-H1, Nδ-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine; sRAGE, soluble receptor of AGEs; SD, standard deviation; HR, hazard ratio; CI, confidence interval; CRC, colorectal cancer.

[a] Cutoffs were based on the distribution of biomarker among all CRC cases (men and women combined).

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

[c] Stratified by center, and adjusted for age at diagnosis (continuous), sex (men, women), tumor stage (I, II, III, IV, missing) location of primary tumor (colon, rectum), smoking status (never, former, current, missing), BMI (kg/m^2; continuous), year of diagnosis (continuous), and diabetes (yes, no, missing).

[d] Likelihood ratio test used for testing interaction.

[e] Calculated with either quintiles or continuous biomarker.

[f] P_{trend} was calculated using the median values of each quintile and adjusting for all the variables of the corresponding model.

[g] One standard deviation for each biomarker was as follows: CML: 1,025 nmol/l, CEL: 775 nmol/l, MG-H1: 261 nmol/l, sRAGE: 475 ng/ml.

[h] Excluded 23 CRC cases with missing sRAGE.

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

Biomarker/ Outcome/ Category	Cut-offs[a]	**Combined**		**Men**		**Women**		
		Deaths/ Total	HR (95% CI)[b,c]	Deaths/ Total	HR (95% CI)[b,c]	Deaths/ Total	HR (95% CI)[b,c]	$p_{interaction}$[c,d,e]
All AGEs (CML+CEL+MG-H1), nmol/L								
CRC-specific mortality								
Quintile 1	≤ 4175	111/270	1.00 (ref)	62/136	1.00 (ref)	49/134	1.00 (ref)	
Quintile 2	[4176-4664]	100/268	1.00 (0.73-1.37)	53/142	0.83 (0.53-1.29)	47/126	0.97 (0.59-1.60)	
Quintile 3	[4665-5166]	106/271	1.15 (0.84-1.58)	53/132	0.98 (0.62-1.54)	53/139	1.20 (0.74-1.96)	
Quintile 4	[5167-6030]	107/266	0.90 (0.65-1.23)	52/120	0.84 (0.53-1.35)	55/146	0.72 (0.43-1.19)	
Quintile 5	≥ 6031	117/264	0.98 (0.70-1.37)	51/116	0.86 (0.53-1.39)	66/148	0.91 (0.54-1.53)	*0.93*
P_{trend}[f]			*0.89*		*0.65*		*0.80*	
Per 1 SD[g]		541/1339	1.03 (0.93-1.14)	271/646	0.96 (0.82-1.12)	270/693	1.13 (0.95-1.34)	*0.33*
All-cause mortality								
Quintile 1	≤ 4175	143/273	1.00 (ref)	79/137	1.00 (ref)	64/136	1.00 (ref)	
Quintile 2	[4176-4664]	144/274	1.07 (0.82-1.40)	80/146	0.93 (0.64-1.35)	64/128	1.07 (0.69-1.64)	
Quintile 3	[4665-5166]	125/274	1.01 (0.76-1.33)	64/134	0.85 (0.57-1.26)	61/140	1.14 (0.74-1.76)	

Quintile 4	[5167-6030]	134/274	0.84 (0.63-1.10)	67/125	0.74 (0.50-1.12)	67/149	0.77 (0.50-1.19)	
Quintile 5	≥ 6031	147/274	0.89 (0.66-1.19)	68/122	0.79 (0.52-1.20)	79/152	0.94 (0.60-1.49)	*0.98*
P_{trend}[f]			*0.35*		*0.31*		*0.70*	
Per 1 SD[g]		693/1369	0.97 (0.88-1.07)	358/664	0.90 (0.79-1.04)	335/705	1.10 (0.94-1.29)	*0.41*
CEL/MG-H1								
CRC-specific mortality								
Quintile 1	≤ 0.92	123/267	1.00 (ref)	69/142	1.00 (ref)	54/125	1.00 (ref)	
Quintile 2	[0.93-1.17]	96/266	0.68 (0.50-0.92)	54/128	0.77 (0.51-1.15)	42/138	0.60 (0.36-1.00)	
Quintile 3	[1.18-1.48]	114/268	0.83 (0.61-1.13)	55/127	0.70 (0.45-1.07)	59/141	0.84 (0.50-1.42)	
Quintile 4	[1.49-1.83]	102/267	0.79 (0.57-1.11)	51/130	0.58 (0.36-0.92)	51/137	0.88 (0.50-1.54)	
Quintile 5	≥ 1.84	106/271	0.91 (0.63-1.31)	42/119	0.68 (0.40-1.17)	64/152	1.00 (0.55-1.81)	*0.64*
P_{trend}[f]			*0.95*		*0.06*		*0.16*	
Per 1 SD[g]		541/1339	0.99 (0.88-1.12)	271/646	0.80 (0.62-1.03)	270/693	1.13 (0.96-1.33)	*0.08*
All-cause mortality								
Quintile 1	≤ 0.92	156/273	1.00 (ref)	92/146	1.00 (ref)	64/127	1.00 (ref)	
Quintile 2	[0.93-1.17]	131/274	0.66 (0.51-0.86)	72/134	0.61 (0.43-0.87)	59/140	0.72 (0.46-1.13)	
Quintile 3	[1.18-1.48]	140/274	0.78 (0.60-1.03)	68/129	0.65 (0.45-0.94)	72/145	0.85 (0.53-1.36)	
Quintile 4	[1.49-1.83]	134/274	0.72 (0.53-0.96)	69/134	0.54 (0.36-0.79)	65/140	0.88 (0.53-1.44)	
Quintile 5	≥ 1.84	132/274	0.87 (0.63-1.20)	57/121	0.66 (0.42-1.04)	75/153	1.02 (0.60-1.74)	*0.72*
P_{trend}[f]			*0.69*		*0.02*		*0.22*	
Per 1 SD[g]		693/1369	0.96 (0.86-1.07)	358/664	0.80 (0.65-0.97)	335/705	1.10 (0.94-1.28)	*0.14*
(CEL+MG-H1)/CML								
CRC-specific mortality								
Quintile 1	≤ 0.70	123/267	1.00 (ref)	67/138	1.00 (ref)	56/129	1.00 (ref)	
Quintile 2	[0.71-0.88]	101/264	0.90 (0.63-1.26)	54/131	0.84 (0.54-1.31)	47/133	0.96 (0.52-1.77)	
Quintile 3	[0.89-1.06]	91/265	0.89 (0.60-1.31)	49/124	0.68 (0.41-1.13)	42/141	1.06 (0.53-2.10)	
Quintile 4	[1.06-1.29]	116/271	1.16 (0.78-1.72)	56/127	0.75 (0.43-1.30)	60/144	1.31 (0.67-2.57)	
Quintile 5	≥ 1.30	110/272	1.06 (0.69-1.62)	45/126	0.59 (0.32-1.11)	65/146	1.49 (0.73-3.05)	*0.60*
P_{trend}[f]			*0.24*		*0.18*		*0.03*	
Per 1 SD[g]		541/1339	1.07 (0.95-1.21)	271/646	0.86 (0.68-1.09)	270/693	1.24 (1.04-1.47)	*0.11*
All-cause Mortality								
Quintile 1	≤ 0.70	151/273	1.00 (ref)	86/143	1.00 (ref)	65/130	1.00 (ref)	
Quintile 2	[0.71-0.88]	137/274	1.00 (0.74-1.34)	71/136	0.93 (0.64-1.36)	66/138	0.91 (0.53-1.54)	
Quintile 3	[0.89-1.06]	129/274	0.97 (0.70-1.34)	73/129	0.90 (0.60-1.36)	56/145	0.89 (0.49-1.60)	
Quintile 4	[1.07-1.29]	145/274	1.19 (0.85-1.67)	70/129	0.89 (0.56-1.41)	75/145	1.15 (0.64-2.07)	
Quintile 5	≥ 1.30	131/274	1.02 (0.71-1.47)	58/127	0.71 (0.42-1.20)	73/147	1.02 (0.54-1.90)	*0.64*
P_{trend}[f]			*0.63*		*0.14*		*0.13*	
Per 1 SD[g]		693/1369	1.03 (0.92-1.15)	358/664	0.87 (0.71-1.06)	335/705	1.16 (0.99-1.35)	*0.20*

Abbreviations: AGES, advanced glycation end products; CML, Nε-(carboxy-methyl)lysine; CEL, Nε-(carboxy-ethyl)lysine; MG-H1, Nδ-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine; SD, standard deviation; HR, hazard ratio; CI: confidence interval; CRC, colorectal cancer

[a] Cutoffs were based on the distribution of biomarker or the ratio among all CRC cases (men and women combined).
[b] Quintile 1 was a reference category in each model.
[c] Stratified by center, and adjusted for age at diagnosis (continuous), sex (men, women), tumor stage (I, II, III, IV, missing) location of primary tumor (colon, rectum), smoking status (never, former, current, missing), BMI (kg/m^2; continuous), year of diagnosis (continuous), and diabetes (yes, no, missing).
[d] Likelihood ratio test used for testing interaction.
[e] Calculated with either quintiles or continuous biomarker.
[f] *P* trend was calculated using the median values and adjusting for all the variables of the corresponding model.
[g] One standard deviation for each biomarker as follows: AGEs: 1,474 nmol/l, CEL/MG-H1: 0.80, (CEL+MG-H1)/CML: 0.39.

Figure 1. Multivariable-adjusted HRs and 95% CIs for one SD change in the sum AGEs (**A**) and sRAGE (**B**) for CRC-specific mortality for sensitivity analyses and across strata of potential effect modifiers in the EPIC study.

Figure 2. 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.

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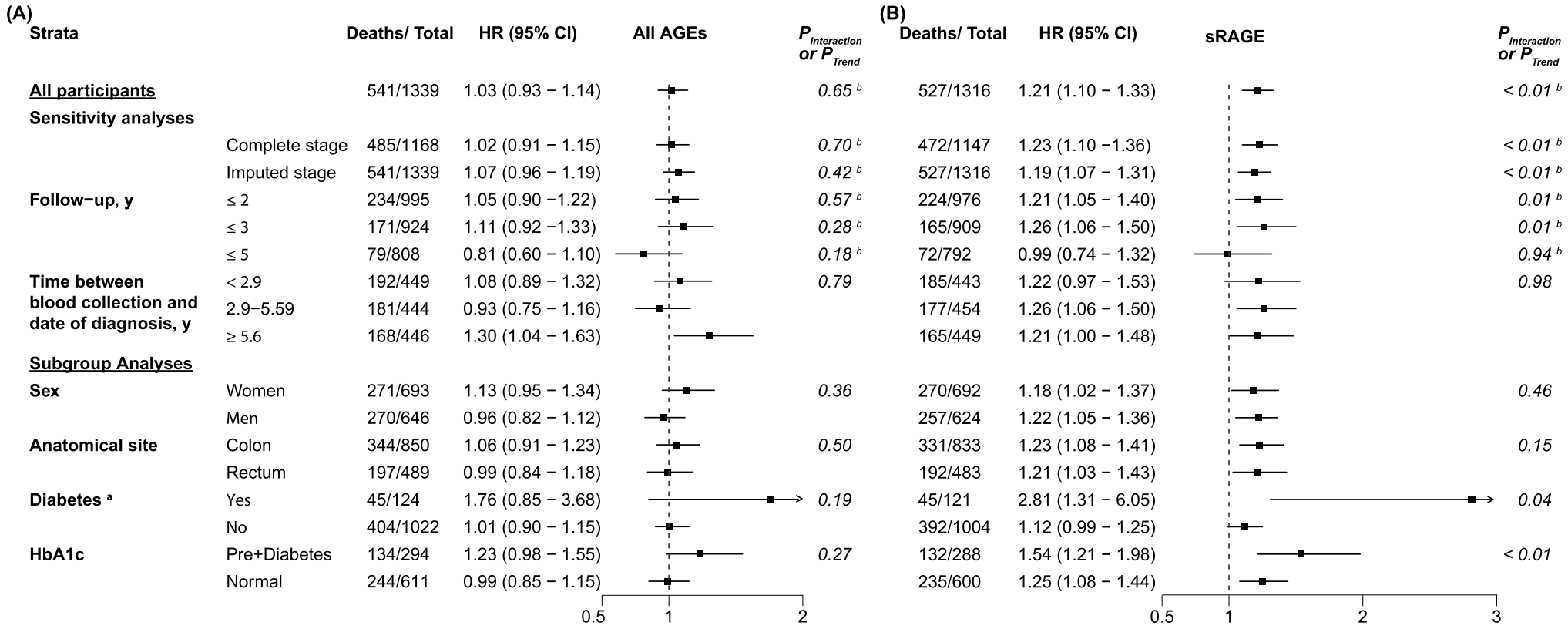
Figure 1. Multivariable-adjusted HRs and 95% CIs for one SD change in the sum AGEs (**A**) and sRAGE (**B**) for CRC-specific mortality for sensitivity analyses and across strata of potential effect modifiers in the EPIC study.

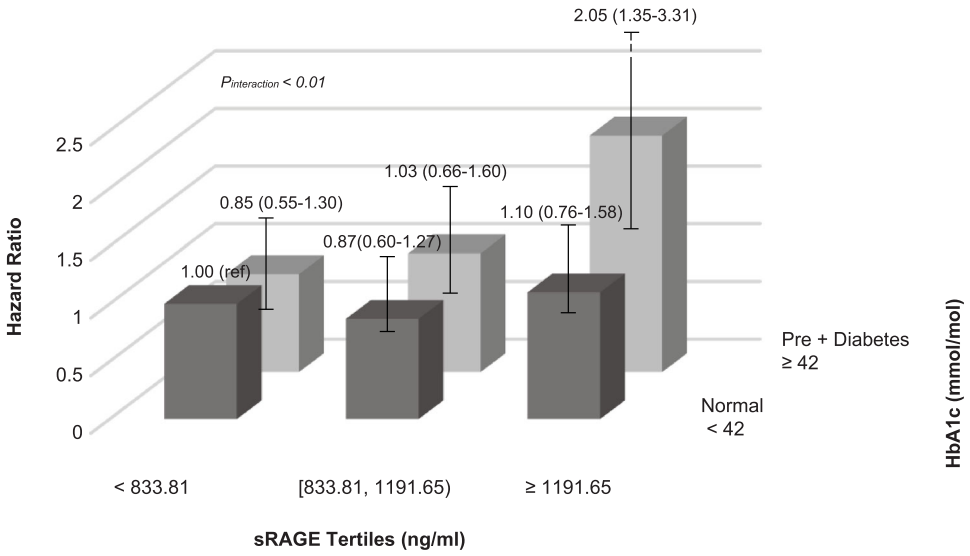


Abbreviations: AGEs, advanced glycation end products; sRAGE, soluble receptor of AGEs; CRC, colorectal cancer; HR, hazard ratio; CI, cofidence interval; y, years.

[a] This category includes self-reported cases of diabetes at baseline and inew incident cases indentified between baseline and cancer diagnosis.

[b] P_{trend}.

Figure 2. 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.



Supplementary Materials

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

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Supplementary 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
CML, mean (SD), nmol/L	1711.46 (197.16)	2123.95 (86.96)	2453.19 (100.90)	2899.71 (164.33)	4314.62 (1076.61)
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)
Men, N (%)	146 (22)	120 (18)	131 (20)	128 (19)	139 (21)
Tumor stage, N (%)[a]					
I	69 (20)	80 (23)	75 (21)	69 (20)	60 (17)
II	65 (24)	48 (18)	56 (21)	48 (18)	54 (20)
III	81 (20)	70 (17)	76 (19)	87 (21)	96 (23)
IV	31 (20)	39 (25)	23 (15)	28 (18)	36 (23)
Colon[b]	196 (23)	178 (20)	180 (21)	171 (20)	145 (17)
Rectum	77 (15)	96 (19)	94 (19)	103 (21)	129 (26)
Year at diagnosis, mean (min-max)	1999 (1993-2010)	1999 (1993-2010)	1999 (1993-2012)	2000 (1994-2008)	2000 (1993-2010)
Smoking status, N (%)[a]					
Never	108 (19)	116 (20)	112 (20)	125 (22)	106 (19)
Former	74 (16)	90 (19)	108 (23)	93 (20)	98 (21)
Current	86 (26)	62 (19)	53 (16)	55 (17)	70 (21)
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)
Inactive	52 (23)	40 (18)	45 (20)	41 (18)	47 (21)
Moderately inactive	81 (21)	67 (17)	76 (20)	69 (18)	92 (24)
Moderately active	108 (20)	120 (22)	109 (20)	113 (21)	96 (18)
Active	25 (20)	25 (20)	25 (20)	22 (17)	30 (24)
Diabetes, N (%)[a,d]					
1	20 (25)	15 (19)	12 (15)	15 (19)	18 (23)
0	239 (22)	219 (20)	202 (19)	212 (20)	215 (20)
Daily dietary intakes					
Dietary fiber, mean (SD), g[e]	21.75 (7.81)	22.58 (7.86)	22.03 (7.54)	23.31 (8.07)	24.61 (8.07)
Dietary calcium, mean (SD), mg[e]	939.91 (408.14)	983.81 (459.20)	979.67 (394.64)	1000.70 (401.49)	1067.02 (439.91)
Vegetables & fruits, mean (SD), g[e]	403.83 (261.49)	401.02 (217.20)	399.61 (230.25)	410.90 (263.93)	375.83 (202.14)
Red & processed meat, mean (SD), g[e]	92.19 (49.42)	85.52 (97.46)	84.01 (54.64)	86.32 (57.55)	94.38 (54.47)
Energy intake, mean (SD), kcal[e]	2186.67 (661.19)	2165.09 (928.80)	2116.49 (678.80)	2081.36 (670.24)	2182.53 (611.36)

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

[a] Total percentage do not add up to 100% because of missing data.

[b] colon cancer: this many proximal, distal, unidentified

[c] Sex-specific categories.

[d] This category includes self-reported cases of type II diabetes at baseline and new incident cases identified between baseline and cancer diagnosis.

[e] Missing values were imputed with sex-specific dietary medians for: dietary fiber (n = 3), energy intake (n = 3).

Supplementary Table 2. Selected baseline characteristics of CRC cases (N = 1,369) according to quintiles of circulating N(epsilon)-(carboxyethyl)lysine(CEL) in the EPIC study.

Baseline characteristic	N(epsilon)-(carboxyethyl)lysine(CEL), nmol/L				
	Quintile 1: ≤ 982 N=273	Quintile 2: 983-1242 N = 274	Quintile 3: 1243-1477 N = 274	Quintile 4: 1478-1785 N = 274	Quintile 5: ≥ 1786 N = 274
CEL, mean (SD), nmol/L	787.53 (149.38)	1122.05 (73.88)	1355.84 (67.97)	1617.47 (86.32)	2494.77 (1137.75)
Age at diagnosis, mean (SD), y	62,84 (6.86)	62.57(8.00)	63.52(7.64)	61.79 (7.44)	62.29 (8.19)
Men, N (%)	144 (22)	137 (21)	136 (20)	121 (18)	126 (19)
Tumor stage, N (%)[a]					
I	60 (14)	87 (25)	79 (22)	73 (21)	64 (18)
II	67 (25)	48 (18)	66 (24)	43 (16)	47 (17)
III	95 (23)	79 (19)	79 (19)	90 (22)	67 (16)
IV	38 (24)	35 (22)	22 (14)	37 (24)	25 (16)
Location of primary tumor, N (%)					
Colon[b]	161 (19)	178 (20)	170 (20)	189 (22)	172 (20)
Rectum	112 (22)	96 (19)	104 (21)	85 (17)	102 (20)
Year at diagnosis, mean (min-max)	1999 (1994-2012)	2000 (1993-2010)	2000 (1993-2010)	1999 (1994-2006)	2000 (1993-2010)
Smoking status, N (%)[a]					
Never	98 (17)	114 (20)	128 (23)	113 (20)	114 (20)
Former	86 (19)	85 (18)	100 (22)	87 (19)	105 (23)
Current	89 (27)	68 (21)	45 (14)	73 (22)	51 (16)
BMI, mean (SD), kg/m^2	28.28 (4.04)	26.69 (4.68)	26.80 (4.26)	26.80 (4.24)	26.94 (4.31)
Physical activity, N (%)[a,c]					
Inactive	51 (23)	42 (19)	43 (19)	50 (22)	39 (17)
Moderately inactive	88 (23)	72 (19)	74 (19)	68 (18)	83 (22)
Moderately active	99 (18)	107 (20)	111 (20)	115 (21)	114 (21)
Active	32 (25)	23 (18)	23 (18)	26 (20)	23 (18)
Diabetes, N (%)[a,d]					
1	14 (18)	16 (20)	15 (19)	16 (20)	19 (24)
0	222 (20)	207 (19)	213 (20)	223 (21)	222 (20)
Daily dietary intakes					
Dietary fiber, mean (SD), g[e]	23.16 (7.83)	22.49 (8.31)	21.98 (7.31)	23.03 (7.09)	23.62 (8.70)
Dietary calcium, mean (SD), mg[e]	1034.56 (439.06)	973.84 (429.91)	985.57 (448.39)	1003.77 (419.25)	973.72 (373.60)
Vegetables & fruits, mean (SD), g[e]	390.66 (244.89)	373.82 (205.28)	375.28 (214.63)	404.33 (218.64)	447.06 (283.15)
Red & processed meat, mean (SD), g[e]	94.33 (52.31)	92.05 (61.64)	83.45 (50.31)	90.79 (51.24)	81.81 (97.33)
Energy intake, mean (SD), kcal[e]	2195.73 (626.51)	2124.31 (693.99)	2055.59 (668.87)	2175.04 (632.35)	2181.51 (925.85)

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

[a] Total percentage do not add up to 100% because of missing data.

[b] colon cancer: this many proximal, distal, unidentified

[c] Sex-specific categories.

[d] This category includes self-reported cases of type II diabetes at baseline and new incident cases identified between baseline and cancer diagnosis.

[e] Missing values were imputed with sex-specific dietary medians for: dietary fiber (n = 3), energy intake (n = 3).

Supplementary Table 3. Selected baseline characteristics of CRC cases (N = 1,369) according to quintiles of circulating Methylglyoxal(MG-H1) in the EPIC study.

Baseline characteristic	Methylglyoxal(MG-H1), nmol/L				
	Quintile 1: ≤ 858 N=273	Quintile 2: 859-953 N = 274	Quintile 3: 954-1058 N = 274	Quintile 4: 1059-1222 N = 274	Quintile 5: ≥ 1223 N = 274
MG-H1, mean (SD), nmol/L	777.46 (65.18)	906.72 (25.58)	1001.56 (29.81)	1126.09 (44.18)	1463.42 (242.12)
Age at diagnosis, mean (SD), y	61.62 (6.82)	63.21(7.40)	63.02(8.47)	62.14 (7.77)	63.01 (7.63)
Men, N (%)	145 (22)	134 (20)	123 (19)	126 (19)	136 (20)
Tumor stage, N (%)[a]					
I	66 (19)	74 (21)	63 (18)	86 (24)	64 (18)
II	67 (25)	54 (20)	48 (18)	48 (18)	54 (20)
III	76 (19)	88 (21)	90 (22)	69 (17)	87 (21)
IV	35 (22)	31 (20)	29 (18)	19 (12)	43 (27)
Location of primary tumor, N (%)					
Colon[b]	192 (22)	182 (21)	171 (20)	158 (18)	167 (19)
Rectum	81 (16)	92 (18)	103 (21)	116 (23)	107 (21)
Year at diagnosis, mean (min-max)	1999 (1993-2010)	1999 (1993-2006)	2000 (1993-2012)	2000 (1994-2010)	2000 (1993-2008)
Smoking status, N (%)[a]					
Never	111 (20)	109 (19)	121 (21)	116 (20)	110 (19)
Former	82 (18)	94 (20)	97 (21)	97 (21)	93 (20)
Current	76 (23)	68 (21)	55 (17)	59 (18)	68 (21)
BMI, mean (SD), kg/m^2	28.58 (4.66)	27.08 (4.47)	26.39 (3.94)	25.99 (4.06)	25.47 (3.68)
Physical activity, N (%)[a,c]					
Inactive	58 (26)	50 (22)	33 (15)	35 (16)	49 (22)
Moderately inactive	71 (18)	71 (18)	82 (21)	75 (19)	86 (22)
Moderately active	112 (21)	116 (21)	124 (23)	104 (19)	90 (16)
Active	26 (20)	22 (17)	20 (16)	28 (22)	31 (24)
Diabetes, N (%)[a,d]					
1	21 (26)	16 (20)	9 (11)	18 (23)	16 (19)
0	230 (21)	233 (21)	210 (19)	199 (18)	215 (20)
Daily dietary intakes					
Dietary fiber, mean (SD), g[e]	22.49 (7.03)	22.20 (8.06)	23.31 (7.60)	22.80 (8.48)	23.49 (8.13)
Dietary calcium, mean (SD), mg[e]	971.93 (423.56)	974.02 (416.36)	975.47 (418.22)	994.19 (404.82)	1055.61 (447.21)
Vegetables & fruits, mean (SD), g[e]	399.81 (225.80)	402.57 (249.09)	411.56 (241.05)	385.35 (223.36)	391.87 (241.45)
Red & processed meat, mean (SD), g[e]	93.07 (56.52)	91.01 (96.44)	86.06 (53.97)	84.22 (54.18)	88.07 (53.80)
Energy intake, mean (SD), kcal[e]	2169.54 (628.70)	2132.34 (870.73)	2173.70 (687.45)	2093.43 (701.20)	2163.08 (685.26)

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

[a] Total percentage do not add up to 100% because of missing data.

[b] colon cancer: this many proximal, distal, unidentified

[c] Sex-specific categories.

[d] This category includes self-reported cases of type II diabetes at baseline and new incident cases identified between baseline and cancer diagnosis.

[e] Missing values were imputed with sex-specific dietary medians for: dietary fiber (n = 3), energy intake (n = 3).

Supplementary Table 4. Selected baseline characteristics of CRC cases (N = 1,346) according to quintile of circulating Soluble Receptor for Advanced Glycation End Products (sRAGE) in the EPIC study.

Baseline characteristic	Soluble Receptor for Advanced Glycation End Products (sRAGE), ng/mL				
	Quintile 1: ≤ 713 N = 269	Quintile 2: 714-904 N = 269	Quintile 3: 905-1103 N = 270	Quintile 4: 1104-1403 N = 269	Quintile 5: ≥ 1404 N = 269
sRAGE, mean(SD), ng/mL	573.1 (105.92)	807.6 (57.25)	999.1 (57.77)	1243.9 (86.56)	1831.2 (417.45)
Age at diagnosis, mean (SD), y	62.7 (7.12)	63.7 (7.33)	62.0 (7.73)	62.1 (8.20)	62.5 (7.96)
Men, N (%)	173 (27)	157 (24)	129 (20)	92 (14)	91 (14)
Tumor stage, N (%)[a]					
I	58 (17)	78 (22)	78 (22)	69 (20)	64 (18)
II	69 (26)	46 (17)	47 (18)	64 (24)	41 (15)
III	80 (20)	80 (20)	80 (20)	73 (18)	92 (23)
IV	33 (22)	35 (23)	34 (23)	26 (17)	23 (15)
Location of primary tumor, N (%)					
Colon	162 (19)	176 (21)	162 (19)	177 (21)	176 (21)
Rectum	107 (22)	93 (19)	108 (22)	92 (19)	93 (19)
Year at diagnosis, mean (min-max)	2000 (1993-2009)	2000 (1993-2005)	2000 (1994-2012)	2000 (1993-2010)	1999 (1993-2010)
Smoking status, N (%)[a]					
Never	93 (17)	87 (16)	120 (21)	121 (22)	140 (25)
Former	105 (23)	103 (23)	92 (20)	81 (18)	73 (16)
Current	69 (22)	75 (24)	56 (18	64 (20)	54 (17)
BMI, mean (SD), kg/m^2	28.1 (4.61)	27.1 (3.97)	26.5 (3.86)	26.1 (4.41)	25.7 (4.20)
Physical activity, N (%)[a,b]					
Inactive	56 (26)	50 (23)	38 (17)	38 (17)	36 (17)
Moderately inactive	85 (22)	71 (19)	73 (19)	75 (20)	75 (20)
Moderately active	92 (17)	102 (19)	114 (21)	111 (21)	120 (22)
Active	28 (22)	33 (26)	25 (20)	22 (17)	19 (15)
Diabetes, N (%)[a,c]					
Yes	18 (23)	17 (22)	20 (26)	8 (10)	14 (18)
No	219 (21)	205 (19)	206 (19)	211 (20)	226 (21)
Daily dietary intakes					
Dietary fiber, mean (SD), g[d]	22.7 (8.42)	22.9 (7.77)	22.2 (7.18)	23.3 (7.95)	23.2 (7.96)
Dietary calcium, mean (SD), mg[d]	927.7 (399.83)	1004.8 (444.83)	992.8 (399.83)	1025.7 (413.92)	1016.0 (438.12)
Vegetables & fruits, mean (SD), g[d]	381.5 (233.51)	377.1 (232.00)	389.5 (233.07)	426.3 (243.99)	423.2 (237.17)
Red & processed meat, mean (SD), g[d]	104.8 (98.13)	94.4 (54.74)	85.4 (53.30)	78.5 (53.76)	76.8 (48.37)
Energy intake, mean (SD), kcal[d]	2191.9 (889.87)	2178.8 (662.87)	2115.8 (670.57)	2157.1 (721.71)	2077.7 (633.00)

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

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

[b] Sex-specific categories.

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

[d] Missing values were imputed with sex-specific dietary medians: Dietary fiber (n = 3), Dietary calcium (n = 450), Vegetables & fruits (n = 3), Red & processed meat (n = 3), Energy intake (n = 3).

Supplementary Table 5. Multivariable-adjusted HRs and 95% CIs for one SD increments of CML, CEL, MG-H1, all AGEs or sRAGE for CRC-specific mortality across strata of effect modifiers the EPIC study.

	CML			CEL		MG-H1		ALL AGEs		sRAGE		
Risk factor	Deaths/ Total	HR (95%CI) [a]	$p_{interaction}$ or p_{trend}	HR (95%CI) [a]	$p_{interaction}$ or p_{trend}	HR (95%CI) [a]	$p_{interaction}$ or p_{trend}	HR (95%CI) [a]	$p_{interaction}$ or p_{trend}	Deaths/ Total	HR (95%CI) [a]	$p_{interaction}$ or p_{trend}
All participants	541/1339	1.01 (0.90-1.13)	*0.93* [d]	1.02 (0.91-1.15)	*0.72* [d]	1.06 (0.96-1.17)	*0.23* [d]	1.03 (0.93-1.14)	*0.65* [d]	527/1316	1.21 (1.10-1.33)	*< 0.01* [d]
Sensitivity analyses												
Complete stage[b]	485/1168	1.00 (0.89-1.13)	*0.97* [d]	1.05 (0.88-1.26)	*0.61* [d]	1.06(0.96-1.17)	*0.25* [d]	1.02 (0.91-1.15)	*0.70* [d]	472/1147	1.23 (1.10-1.36)	*< 0.01* [d]
Imputed stage[c]	541/1339	1.02 (0.91-1.15)	*0.67* [d]	1.11 (0.98-1.26)	*0.54* [d]	1.06 (0.97-1.17)	*0.17* [d]	1.07 (0.96-1.19)	*0.42* [d]	527/1316	1.19 (1.07-1.31)	*< 0.01* [d]
Time between blood collection and date of diagnosis, y												
< 2.9	192/449	1.01 (0.81-1.25)	*0.73*	1.14 (0.95-1.35)	*0.68*	1.04 (0.85-1.26)	*0.11*	1.08 (0.89-1.32)	*0.79*	185/443	1.22 (0.97-1.53)	*0.98*
2.9-5.59	181/444	0.94 (0.75-1.19)		0.88 (0.66-1.17)		1.03 (0.85-1.25)		0.93 (0.75-1.16)		177/454	1.26 (1.06-1.50)	
≥ 5.6	168/446	1.32 (1.04-1.68)		1.06 (0.81-1.38)		1.36 (1.11-1.66)		1.30 (1.04-1.63)		165/449	1.21 (1.00-1.48)	
Follow-up[e], y												
≥ 2	234/995	1.03 (0.86-1.22)	*0.76* [d]	1.00 (0.84-1.19)	*0.99* [d]	1.14 (1.00-1.31)	*0.05* [d]	1.05 (0.90-1.22)	*0.57* [d]	224/976	1.21 (1.05-1.40)	*0.01* [d]
≥ 3	171/924	1.07 (0.88-1.30)	*0.49* [d]	1.08 (0.86-1.36)	*0.49* [d]	1.16 (0.99-1.36)	*0.06* [d]	1.11 (0.92-1.33)	*0.27* [d]	165/909	1.26 (1.06-1.50)	*0.01* [d]
≥ 5	79/808	0.82 (0.59-1.14)	*0.23* [d]	0.80 (0.55-1.16)	*0.24* [d]	0.96 (0.73-1.26)	*0.77* [d]	0.81 (0.60-1.10)	*0.18* [d]	73/792	0.99 (0.74-1.32)	*0.94* [d]
Subgroup Analyses												
Sex												
Women	271/693	1.00 (0.82-1.21)	*0.06*	1.19 (1.01-1.40)	*0.74*	1.15 (0.98-1.35)	*0.33*	1.13 (0.95-1.34)	*0.36*	270/692	1.18 (1.02-1.37)	*0.46*
Men	270/646	1.02 (0.87-1.20)		0.82 (0.66-1.03)		0.99 (0.86-1.14)		0.96 (0.82-1.12)		257/624	1.22 (1.05-1.36)	
Age at diagnosis, y												
< 62.84	257/681	0.99 (0.84-1.17)	*0.34*	1.09 (0.88-1.34)	*0.97*	1.03 (0.90-1.19)	*0.44*	1.03 (0.88-1.20)	*0.42*	249/669	1.18 (1.02-1.37)	*0.75*
≥ 62.84	284/658	1.03 (0.87-1.21)		0.98 (0.84-1.14)		1.05 (0.91-1.21)		1.01 (0.87-1.17)		278/647	1.21 (1.06-1.38)	
Anatomical site												
Colon	344/850	0.99 (0.84-1.17)	*0.97*	1.11 (0.96-1.29)	*0.17*	1.04 (0.91-1.20)	*0.81*	1.06 (0.91-1.23)	*0.50*	331/833	1.23 (1.08-1.41)	*0.15*
Rectum	197/489	0.99 (0.83-1.19)		0.95 (0.73-1.23)		1.07 (0.91-1.25)		0.99 (0.84-1.18)		192/483	1.21 (1.03-1.43)	
Colon subsite[f]												
Proximal	156/410	1.02 (0.79-1.32)	*0.50*	0.99 (0.73-1.36)	*0.51*	1.21 (0.97-1.51)	*0.85*	1.05 (0.82-1.34)	*0.35*	148/399	1.15 (0.89-1.47)	*1.00*
Distal	150/363	1.02 (0.77-1.34)		1.05 (0.77-1.43)		0.97 (0.76-1.24)		1.02 (0.79-1.33)		149/358	1.19 (0.95-1.49)	
Stage[g]												
I and II	134/605	1.07 (0.84-1.35)	*0.62*	1.14 (0.81-1.59)	*0.79*	1.17 (0.96-1.42)	*0.33*	1.12 (0.89-1.42)	*0.48*	131/595	1.17 (0.94-1.45)	*0.12*
III and IV	351/563	0.97 (0.84-1.12)		0.98 (0.78-1.24)		1.01 (0.89-1.14)		0.98 (0.85-1.13)		341/552	1.25 (1.10-1.43)	
Year of diagnosis												
< 2000	264/649	0.93 (0.78-1.10)	*0.94*	1.07 (0.92-1.25)	*0.96*	0.95 (0.82-1.10)	*0.12*	0.97 (0.84-1.14)	*0.86*	254/633	1.21 (1.04-1.41)	*0.78*
≥ 2000	277/690	1.06 (0.89-1.27)		0.93 (0.76-1.15)		1.16 (1.00-1.35)		1.05 (0.89-1.24)		273/683	1.13 (0.98-1.31)	
BMI, kg/m^2												
< 25	197/506	1.08 (0.89-1.30)	*0.81*	1.08 (0.90-1.26)	*0.13*	1.21 (1.02-1.43)	*0.36*	1.11 (0.94-1.31)	*0.65*	195/502	1.05 (0.88-1.26)	*0.24*
25-29.9	238/587	1.00 (0.83-1.21)		0.99 (0.76-1.30)		0.98 (0.83-1.16)		0.99 (0.83-1.19)		227/574	1.30 (1.10-1.42)	
≥ 30	106/246	1.17 (0.79-1.73)		0.91 (0.49-1.67)		1.04 (0.73-1.50)		1.11 (0.73-1.54)		105/240	1.42 (1.05-1.91)	
Physical activity												
Inactive	70/196	0.91 (0.57-1.48)	*0.29*	1.13 (0.62-2.07)	*0.15*	1.01 (0.70-1.47)	*0.03*	0.98 (0.64-1.52)	*0.08*	66/190	0.86 (0.52-1.42)	*0.27*
Moderately inactive	150/369	0.99 (0.81-1.21)		0.93 (0.73-1.19)		0.84 (0.68-1.05)		0.94 (0.78-1.15)		145/362	1.51 (1.20-1.90)	
Moderately active	239/557	1.13 (0.91-1.41)		1.04 (0.88-1.24)		1.36 (1.15-1.62)		1.14 (0.96-1.36)		235/550	1.18 (1.01-1.37)	

Active	47/132	1.21 (0.60-2.46)		2.65 (1.04-6.78)		1.32 (0.71-2.46)		1.61 (0.81-3.23)		47/132	1.83 (0.94-3.56)	
Diabetes[h]												
Yes	45/124	1.45 (0.74-2.84)	*<0.01*	1.88 (0.69-5.16)	*0.22*	2.12 (1.02-4.41)	*0.07*	1.76 (0.85-3.68)	*0.19*	45/121	2.81 (1.31-6.05)	*0.04*
No	404/1022	0.95 (0.83-1.09)		1.10 (0.96-1.26)		1.03 (0.92-1.15)		1.01 (0.90-1.15)		392/1004	1.12 (0.99-1.25)	
HbA1c[j]												
Pre+Diabetes	134/294	1.23 (0.98-1.55)	*0.50*	1.08 (0.70-1.67)	*0.20*	1.22 (0.99-1.50)	*0.56*	1.23 (0.98-1.55)	*0.27*	132/288	1.54 (1.21-1.98)	*< 0.01*
Normal	244/611	0.99 (0.83-1.17)		0.97 (0.82-1.15)		1.03 (0.89-1.20)		0.99 (0.85-1.15)		235/600	1.25 (1.08-1.44)	
Fasting Status												
No	270/679	1.05 (0.91-1.20)	0.03	1.07 (0.92-1.25)	0.50	1.05 (0.93-1.19)	0.85	1.06 (0.93-1.21)	0.28	263/648	1.28(1.11-1.47)	0.53
In Between	118/277	0.95 (0.69-1.31)		1.09 (0.76-1.55)		1.08 (0.84-1.37)		1.02 (0.76-1.36)		113/263	1.31 (0.98-1.74)	
Yes	142/392	0.69 (0.41-1.15)		0.99 (0.68-1.44)		0.95 (0.71-1.27)		0.82 (0.55-1.24)		140/384	0.99 (0.77-1.29)	

Abbreviations: HR, hazard ratio; y, years; BMI, body mass index; HbA1c, glycosylated hemoglobin; CML, Nε-(carboxy-methyl)lysine; CEL, Nε-(carboxy-ethyl)lysine (CEL); MG-H1, Nδ-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine; sRAGE, soluble receptor for AGEs

[a] Multivariable-adjusted model, adjusted for age, sex(combined), stage, tumor location, year of diagnosis, BMI, prevalent diabetes, smoking status, stratified by center. P for interaction is provided unless otherwise indicated.

[b] Excludes missing stage N = 178

[c] Impute missing stage by multiple imputation

[d] p_{trend}

[e] Multivariable model including all CRC cases with time interval between age at DX and age at end of follow up, of more than 2,3, and 5 years.

[f] Only colon tumors with known locations included. Unspecified and overlapping (N = 73) were excluded.

[g] Missing stage variables were not included

[h] This category includes prevalent cases of type II diabetes at baseline and new incident cases identified between baseline and cancer diagnosis. [j] Missing HbA1c not included (N = 445); Normal < 42mmol/mol, Pre+Diabetes ≥ 42mmol/mol

Note: The criteria for separating the continuous variables is based on their medians (including patients with missing sRAGE)

Supplementary Table 6. Multivariable-adjusted HRs and 95% CIs for one SD increments of CML, CEL, MG-H1 all AGEs or sRAGE for overall mortality across strata of effect modifiers in the EPIC study.

		CML		CEL		MG-H1		ALL AGEs		sRAGE		
ity analysis or stratifyin	Deaths/ Total	HR (95%CI) [a]	$p_{interaction}$ or p_{trend}	HR (95%CI) [a]	$p_{interaction}$ or p_{trend}	HR (95%CI) [a]	$p_{interaction}$ or p_{trend}	HR (95%CI) [a]	$p_{interaction}$ or p_{trend}	Deaths/ Total	HR (95%CI) [a]	$p_{interaction}$ or p_{trend}
All participants	693/1369	0.97(0.87-1.08)	*0.58* [d]	0.96 (0.86-1.07)	*0.44* [d]	1.02 (0.93-1.11)	*0.69* [d]	0.97 (0.88-1.07)	*0.51* [d]	677/1346	1.14 (1.04-1.25)	*< 0.01* [d]
Sensitivity analyses												
Complete stage[b]	610/1191	0.96 (0.86-1.07)	*0.42* [d]	0.99 (0.84-1.17)	*0.94* [d]	1.01 (0.92-1.11)	*0.82* [d]	0.97 (0.87-1.08)	*0.60* [d]	595/1170	1.16 (1.06-1.28)	*< 0.01* [d]
Imputed stage[c]	693/1369	0.98 (0.88-1.09)	*0.83* [d]	1.02 (0.91-1.14)	*0.72* [d]	1.02 (0.94-1.11)	*0.61* [d]	1.00 (0.91-1.10)	*0.83* [d]	677/1346	1.12 (1.02-1.22)	*< 0.01* [d]
Time between blood collection and date of diagnosis, y												
< 2.9	241/456	1.02 (0.84-1.25)	*0.95*	1.07 (0.91-1.26)	*0.86*	1.03 (0.86-1.22)	*0.16*	1.06 (0.89-1.26)	*0.83*	232/443	1.20 (0.98-1.47)	*0.89*
2.9-5.59	232/457	0.88 (0.71-1.07)		0.81 (0.62-1.05)		0.94 (0.79-1.12)		0.85 (0.70-1.03)		228/454	1.22 (1.04-1.43)	
≥ 5.6	220/456	1.27 (1.03-1.57)		1.02 (0.80-1.31)		1.29 (1.07-1.54)		1.24 (1.01-1.52)		217/449	1.09 (0.91-1.29)	
Follow-up[e], y												
≥ 2	350/1,022	0.98 (0.85-1.13)	*0.79* [d]	0.94 (0.80-1.09)	*0.41* [d]	1.06 (0.95-1.20)	*0.30* [d]	0.98 (0.85-1.12)	*0.71* [d]	338/1003	1.12 (0.99-1.27)	*0.08* [d]
≥ 3	280/951	1.02 (0.87-1.20)	*0.78* [d]	0.99 (0.82-1.21)	*0.95* [d]	1.08 (0.95-1.23)	*0.23* [d]	1.03 (0.89-1.20)	*0.69* [d]	272/936	1.15 (1.00-1.33)	*0.06* [d]
≥ 5	167/832	0.89 (0.72-1.11)	*0.32* [d]	0.87 (0.66-1.14)	*0.30* [d]	0.98 (0.81-1.18)	*0.82* [d]	0.89 (0.72-1.09)	*0.26* [d]	159/817	0.96 (0.78-1.18)	*0.68* [d]
Subgroup Analyses												
Sex												
Women	335/705	1.02 (0.86-1.22)	*0.08*	1.14 (0.98-1.32)	*0.82*	1.09 (0.95-1.26)	*0.38*	1.10 (0.94-1.29)	*0.44*	335/704	1.15 (1.00-1.31)	*0.44*
Men	358/664	0.97 (0.84-1.13)		0.79 (0.65-0.95)		0.97 (0.86-1.10)		0.90 (0.79-1.04)		342/642	1.16 (1.01-1.33)	
Age at diagnosis, y												
< 62.84	300/686	1.00 (0.86-1.16)	*0.04*	1.07 (0.88-1.30)	*0.72*	1.01 (0.88-1.14)	*0.21*	1.02 (0.89-1.18)	*0.07*	291/674	1.17 (1.01-1.34)	*0.85*
≥ 62.84	393/683	0.96 (0.83-1.11)		0.90 (0.78-1.03)		1.01 (0.89-1.14)		0.93(0.81-1.05)		386/672	1.11 (0.99-1.24)	
Anatomical tumor site												
Colon	447/870	0.92 (0.79-1.07)	*0.35*	1.02 (0.90-1.16)	*0.08*	0.98 (0.87-1.11)	*0.32*	0.96 (0.84-1.10)	*0.97*	432/853	1.15 (1.02-1.29)	*0.57*
Rectum	246/499	0.97 (0.82-1.14)		0.88 (0.70-1.13)		1.05 (0.90-1.21)		0.96 (0.82-1.13)		241/493	1.16 (0.99-1.35)	
Colon tumor subsite[f]												
Proximal	200/419	0.98 (0.78-1.23)	*0.52*	0.96 (0.73-1.27)	*0.52*	1.12 (0.93-1.40)	*0.78*	1.00 (0.81-1.24)	*0.18*	192/408	1.11 (0.90-1.37)	*0.82*
Distal	199/374	0.94 (0.74-1.31)		1.02 (0.78-1.33)		0.88 (0.71-1.08)		0.94 (0.75-1.19)		197/369	1.11 (0.91-1.36)	
Tumor stage[g]												
I and II	198/624	1.03 (0.85-1.25)	*0.41*	1.10 (0.83-1.45)	*0.83*	1.06 (0.90-1.26)	*0.77*	1.07 (0.88-1.29)	*0.44*	193/614	1.16 (0.97-1.38)	*0.15*
III and IV	412/567	0.95 (0.83-1.08)		0.93 (0.74-1.15)		0.99 (0.88-1.12)		0.94 (0.82-1.08)		402/556	1.19 (1.05-1.34)	
Year of diagnosis												
< 2000	342/668	0.93 (0.80-1.09)	*0.47*	1.00 (0.86-1.16)	*0.81*	0.94 (0.83-1.08)	*0.37*	0.95 (0.82-1.09)	*0.86*	330/652	1.17 (1.02-1.34)	*0.62*
≥ 2000	351/701	0.99 (0.84-1.17)		0.89 (0.74-1.07)		1.09 (0.95-1.25)		0.97 (0.84-1.13)		347/694	1.05 (0.92-1.19)	
BMI, kg/m^2												
< 25	250/519	1.07 (0.90-1.26)	*0.99*	1.00 (0.85-1.17)	*0.22*	1.16 (1.00-1.35)	*0.31*	1.07 (0.91-1.24)	*0.62*	248/515	1.04 (0.89-1.23)	*0.68*
25-29.9	300/600	0.94 (0.79-1.11)		0.97 (0.76-1.23)		0.96 (0.82-1.11)		0.94 (0.79-1.11)		289/587	1.17 (1.01-1.37)	
≥ 30	143/250	1.05 (0.76-1.45)		0.81 (0.49-1.32)		0.98 (0.73-1.31)		0.98 (0.70-1.36)		140/244	1.28 (0.99-1.64)	
Physical activity												
Inactive	83/198	0.75 (0.49-1.14)	*0.11*	1.03 (0.60-1.74)	*0.58*	0.86 (0.62-1.20)	*0.02*	0.82 (0.56-1.20)	*0.06*	78/192	0.76 (0.50-1.15)	*0.11*
Moderately inactive	209/382	0.88 (0.73-1.05)		0.91 (0.71-1.15)		0.80 (0.66-0.98)		0.85 (0.71-1.02)		203/375	1.26 (1.05-1.52)	

Moderately active	298/567	1.09 (0.90-1.33)		0.96 (0.82-1.12)		1.25 (1.07-1.46)		1.06 (0.90-1.24)		294/560	1.15 (1.00-1.32)	
Active	66/136	1.84 (1.10-3.08)		1.34 (0.67-2.67)		1.37 (0.85-2.18)		1.79 (1.06-3.04)		66/136	1.53 (0.93-2.52)	
Diabetes[h]												
Yes	65/127	1.22 (0.78-1.92)	*< 0.01*	1.18 (0.61-2.27)	*0.14*	1.29 (0.85-1.98)	*0.15*	1.21 (0.71-2.08)	*0.30*	63/124	1.81 (1.13-2.91)	*0.02*
No	488/1032	0.95 (0.84-1.07)		1.03 (0.92-1.17)		1.01 (0.91-1.12)		0.99 (0.88-1.10)		476/1014	1.06 (0.95-1.18)	
HbA1c[j]												
Normal	175/301	1.23 (1.00-1.52)	*0.34*	1.00 (0.70-1.43)	*0.21*	1.17 (0.97-1.41)	*0.47*	1.20 (0.97-1.48)	*0.17*	171/295	1.42 (1.14-1.75)	*<0.01*
Pre+Diabetes	302/623	0.95 (0.82-1.12)		0.92 (0.78-1.08)		1.00 (0.87-1.15)		0.94 (0.82-1.08)		293/612	1.17 (1.02-1.33)	
Fasting												
No	349/679	1.03 (0.90-1.17)	0.02	0.96 (0.84-1.11)	0.19	1.01 (0.90-1.14)	0.88	1.00 (0.89-1.13)	0.40	341/669	1.22 (1.07-1.40)	0.39
In Between	158/277	0.86 (0.65-1.11)		1.02 (0.77-1.36)		1.02 (0.83-1.25)		0.93 (0.73-1.18)		152/269	1.04 (0.83-1.31)	
Yes	173/392	0.71 (0.45-1.12)		1.00 (0.72-1.40)		0.93 (0.72-1.21)		0.84 (0.58-1.20)		171/387	1.00 (0.79-1.27)	

Abbreviations: HR, hazard ratio; y, years; BMI, body mass index; HbA1c, glycosylated hemoglobin; CML, Nε-(carboxy-methyl)lysine; CEL, Nε-(carboxy-ethyl)lysine (CEL); MG-H1, Nδ-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine; sRAGE, soluble receptor for AGEs

[a] Multivariable-adjusted model, adjusted for age, sex(combined), stage, tumor location, year of diagnosis, BMI, prevalent diabetes, smoking status, stratified by center. P for interaction is provided unless otherwise indicated.

[b] Excludes missing stage N = 178

[c] Impute missing stage by multiple imputation

[d] p_{trend}

[e] Multivariable model including all CRC cases with time interval between age at DX and age at end of follow up, of more than 2,3, and 5 years.

[f] Only colon tumors with known locations included. Unspecified and overlapping (N = 73) were excluded.

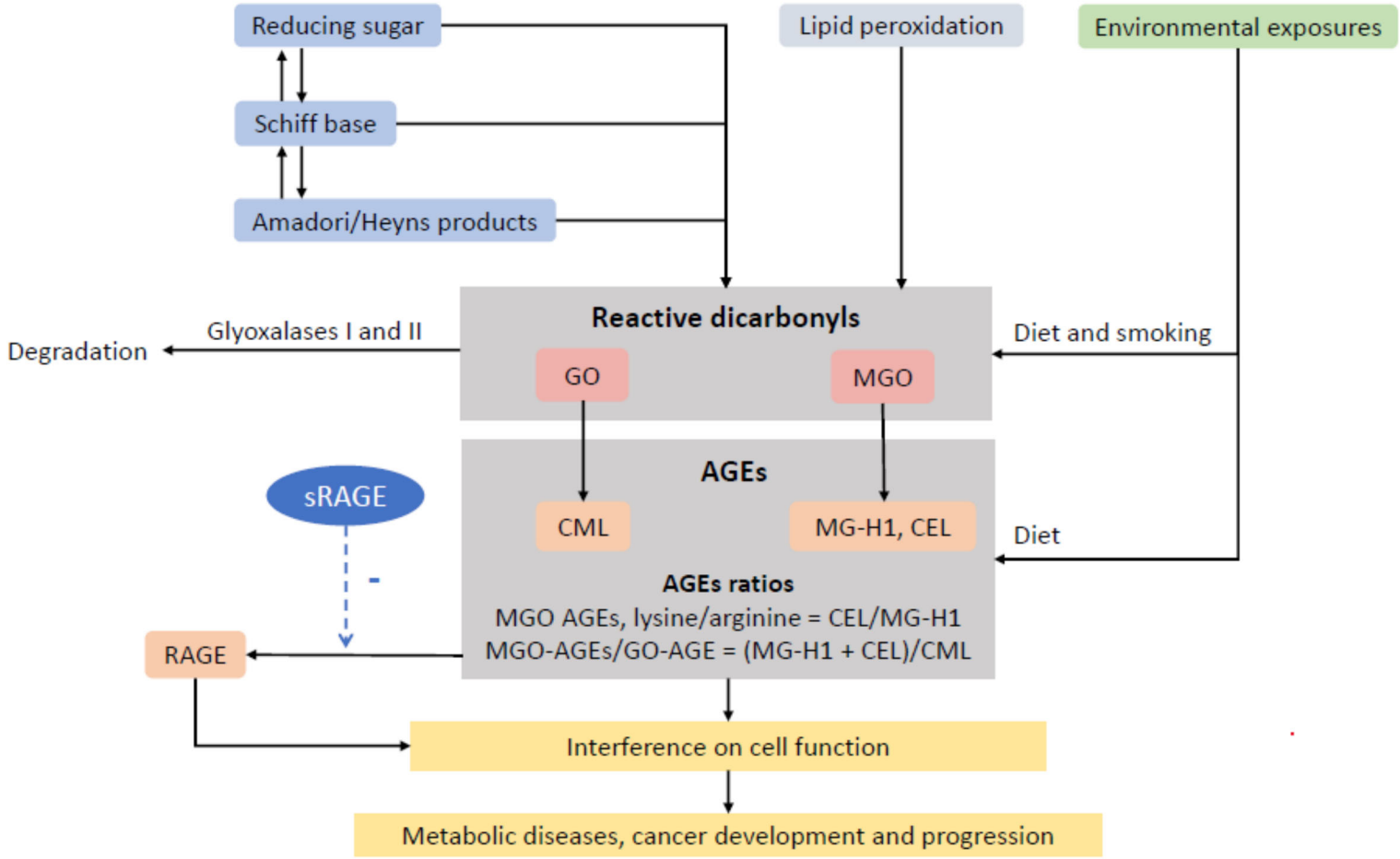
[g] Missing stage variables were not included

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

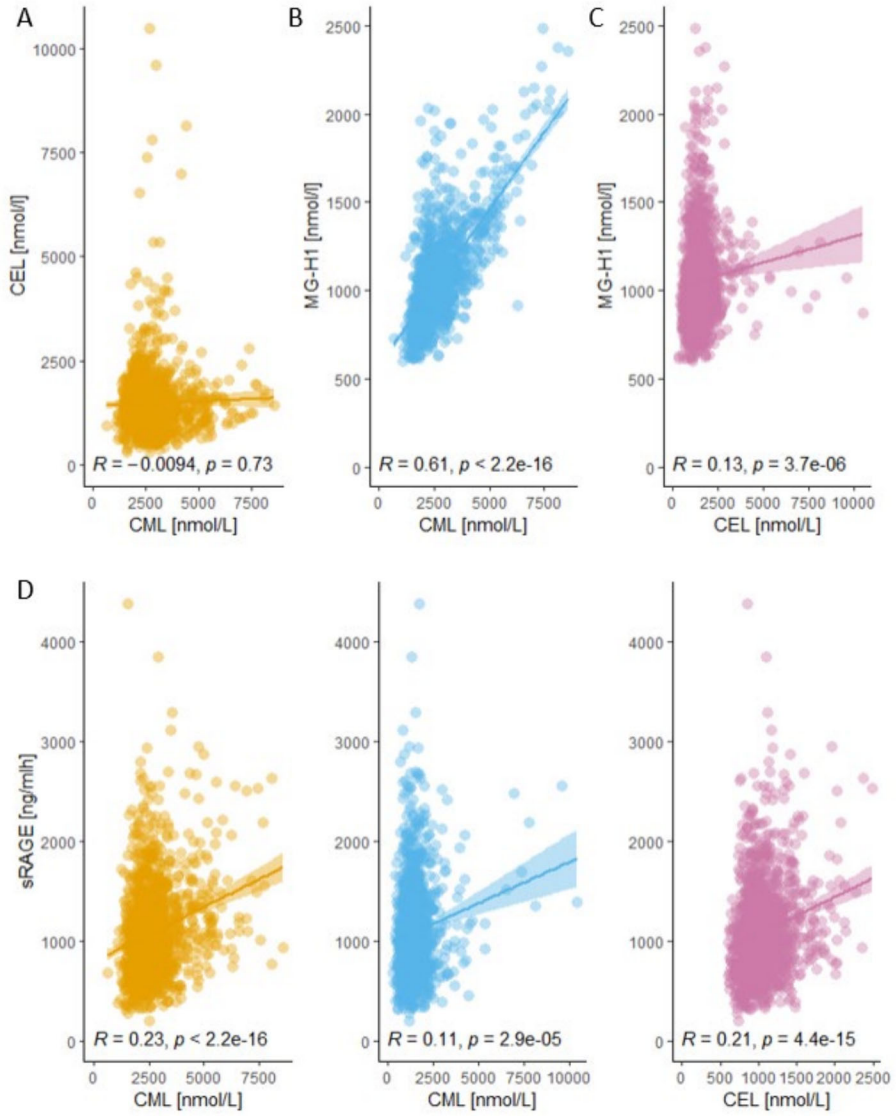
[j] Missing HbA1c not included (N = 445); Categories defined as Normal < 42mmol/mol and Pre+Diabetes ≥ 42mmol/mol

Note: The criteria for separating the continuous variables is based on their medians (including patients with missing sRAGE)

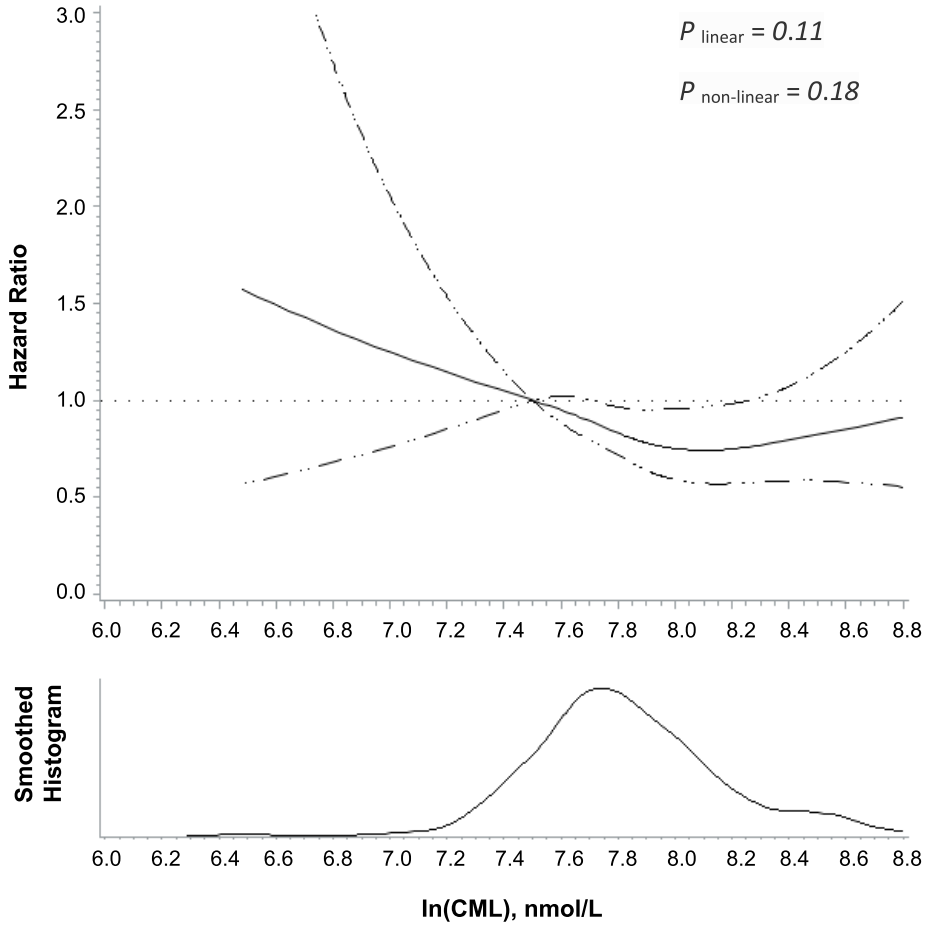
Supplementary Figure 1. Schematic representation of the formation of the AGEs and the rationale for the calculation of the ratio



Supplementary Figure 2. Spearman's correlations between: (A) CEL and CML. (B) MG-H1 and CML. (C) MG-H1 and CEL. (D) sRAGE and other biomarkers.

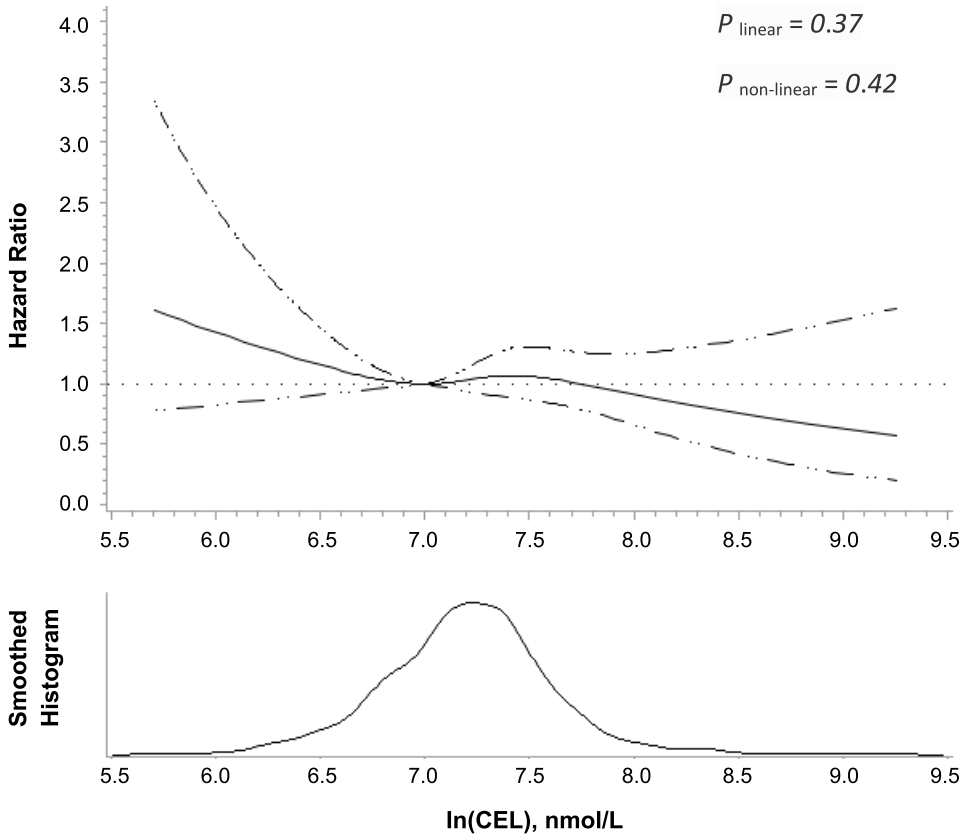


Supplementary Figure 3. Spline regression model for concentration of natural log-transformed CML (nmol/L) and all-cause mortality. Reference 7.5 nmol/L. *Solid line*- HR, *dashed lines*- 95 % CI.[1]

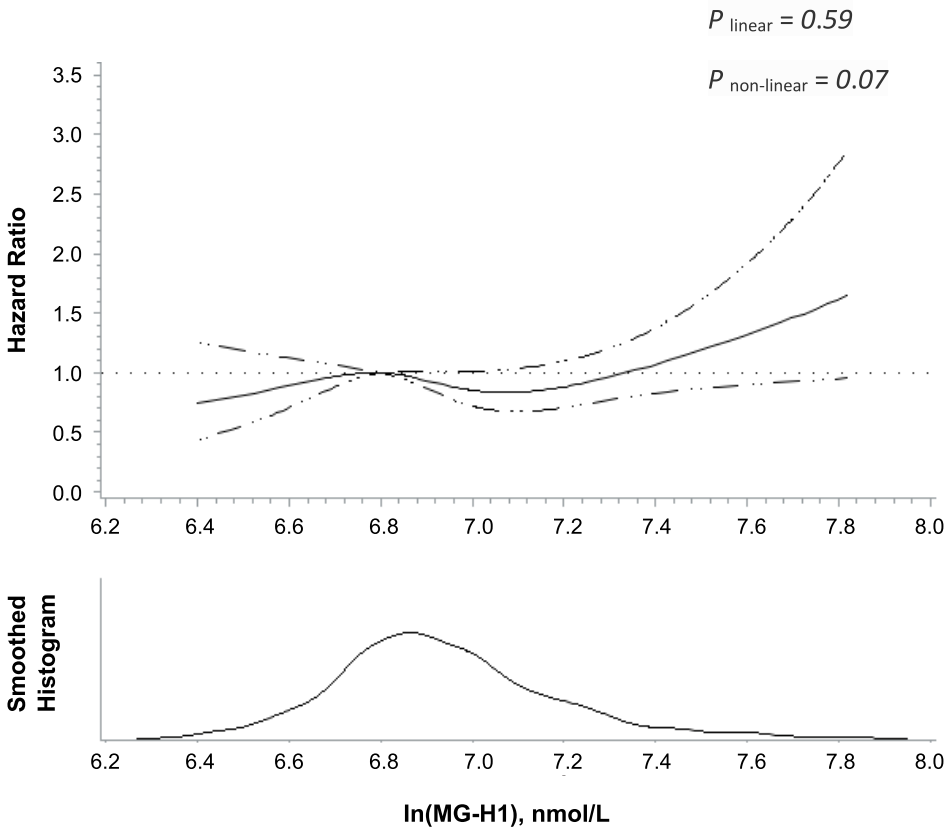


[1] Extreme outliers removed to improve stability of dose response association.

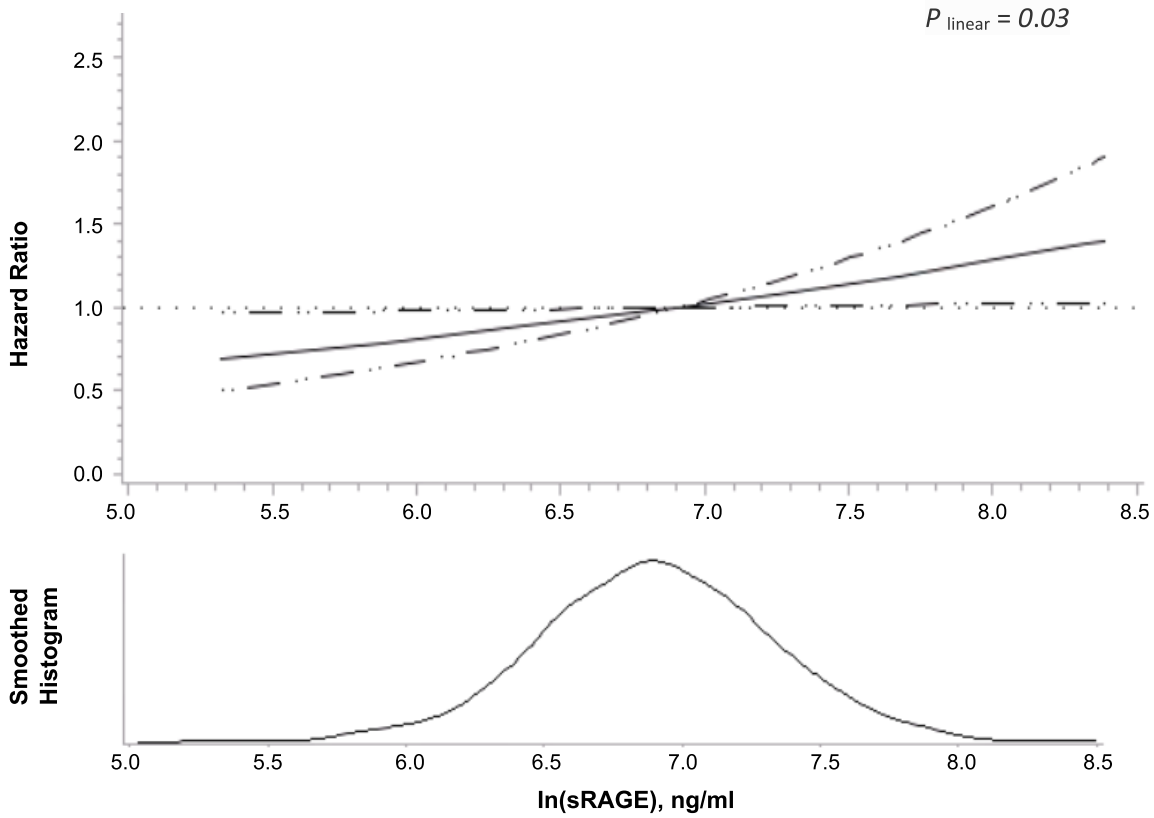
Supplementary Figure 4. Spline regression model for concentration of natural log-transformed CEL (nmol/L) and all-cause mortality. Reference 7 nmol/L. *Solid line*- HR, *dashed lines*- 95 % CI. $P_{linear} = 0.37$. $P_{non\text{-}linear} = 0.42$.



Supplementary Figure 5. Spline regression model for concentration of natural log-transformed MG-H1 (nmol/L) and all-cause mortality. Reference 6.8 nmol/L. *Solid line*- HR, *dashed lines*- 95 % CI. $P_{linear} = 0.59$. $P_{non\text{-}linear} = 0.07$.



Supplementary Figure 6. Spline regression model for concentration of natural log-transformed sRAGE (ng/ml) and all-cause mortality consistent with a linear association. Reference 6.9 nmol/L. *Solid line*- HR, *dashed lines*- 95 % CI. $P_{linear} = 0.03$



Supplementary Figure 7. Multivariable-adjusted HRs and 95% CIs for HbA1c categories (normal and pre-/diabetes) and sRAGE concentration tertiles and all-cause mortality in the EPIC study.