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A prospective study of soluble receptor for advanced glycation end-products and colorectal cancer risk in postmenopausal women

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Conflict of interest

None.

Authorship contribution

Liang Chen, Li Jiao—Conception and design, acquisition of data, experiment, data analysis and interpretation, drafting and revising article, final approval.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.canep.2016.04.004>

Abstract

Objectives—Receptor for advanced glycation end products (RAGE) expressed on adipocytes and immune cells can bind to ligand *N*^ε-(carboxymethyl)-lysine (CML) and trigger dysregulation of adipokines and chronic inflammation. Soluble RAGE (sRAGE) mitigates the detrimental effect of RAGE. We examined the associations between circulating levels of CML-AGE and sRAGE and colorectal cancer (CRC).

Methods—In a case-cohort study of the Women's Health Initiative Study, blood levels of CML-AGE and sRAGE were measured using ELISA. We used multivariable Cox regression model to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) of incident CRC in relation to quartiles (Q) of biomarker levels.

Results—Average follow-up was 7.8 years for 444 cases and 805 subcohort members. In the subcohort, CML-AGE and sRAGE were inversely correlated with BMI (*P* values < 0.0001). Levels of CML-AGE and sRAGE were not associated with CRC. In BMI-specific analysis, the association between sRAGE and CRC was observed. Among women with BMI ≥ 25 kg/m², those with highest levels of sRAGE had significantly lower risk for CRC as compared to women with lowest levels of sRAGE (HR_{Q4 versus Q1}: 0.39; 95% CI: 0.17–0.91). This inverse association was not observed among women with BMI < 25 kg/m² (*P* value for interaction = 0.01).

Conclusions—Among postmenopausal women, the RAGE pathway may be involved in obesity-related CRC.

Keywords

Colorectal cancer; Advanced glycation end-products; Receptor for advanced glycosylation endproducts; Epidemiology; sRAGE; Body weight; Obesity; *N*^ε-(carboxymethyl)-lysine; Pattern recognition receptors

1. Introduction

Colorectal cancer (CRC) is a leading cause of cancer-related deaths in men and women in most industrialized countries. Insulin resistance, excessive adiposity, and chronic inflammation are interrelated mechanisms contributing to CRC development [1]. Receptor for advanced glycation end-products (RAGE) can bind to advanced glycation end-products (AGEs) and trigger dysregulation of adipokines and chronic inflammation.

Advanced Glycation End products (AGEs) are heterogeneous compounds formed irreversibly *via* non-enzymatic glycation and oxidation reactions between reducing sugars (e.g., glucose and fructose) and free amino acid groups on lipids, proteins and nucleotides endogenously as byproducts of normal metabolism [4,5], or exogenously during high temperature cooking practice and from tobacco smoke [2,3]. High consumption of red meat, which is a rich source of AGEs, has been associated with increased CRC risk [1]. The principal mechanism by which AGEs exert their biological function is through interacting with the full-length multi-ligand RAGE (AGER in HUGO nomenclature), which is present on adipocytes, immune, epithelial, and endothelial cells [6,7]. Under normal physiological states, RAGE is expressed at a low level. However, under pathological stress, upregulation of

the RAGE gene is observed in companion with the accumulation of its ligands [8,9]. The interaction between RAGE and its ligand results in downstream activation of the pro-inflammatory transcription factor NF- κ B [11,12], and has been shown to contribute to insulin resistance and chronic inflammation [9,13–15]. *N*^ε-(carboxymethyl)-lysine (CML)-AGE is one of RAGE ligands and the best characterized AGEs [10]. In addition, accumulation of CML-AGE in the adipose tissue has been shown to contribute to dysregulation of adipokines [16]. A previous study found moderate-to-strong expression of CML-AGE in colon adenocarcinoma tissues [17]. There is also experimental evidence for the involvement of the AGEs/RAGE axis in colon inflammation and tumorigenesis [18–22].

Soluble RAGE (sRAGE) includes a C-truncated endogenous secretory isoform of RAGE (esRAGE); as well as cleavage forms of esRAGE and membrane-bound full-length RAGE [23,24]. sRAGE binds to RAGE ligands without triggering a signaling cascade because it has no transmembrane and intracellular domains. Therefore, sRAGE may be an endogenous factor that protects against AGEs/RAGE-mediated events [25,26].

We proposed that the AGEs/RAGE axis contributes the biological connection between environmental risk factors (such as red meats) and suggestive etiological mechanisms for CRC [12,31]. In this prospective study, we examined the associations between prediagnostic levels of CML-AGE and sRAGE and risk of subsequent development of CRC in the Women's Health Initiative Observational Study (WHI-OS). We hypothesized that higher baseline levels of CML-AGE and lower levels of sRAGE would be associated with an increased CRC risk, and that the association would differ according to baseline levels of BMI status, insulin and C-reactive protein (CRP).

2. Materials and methods

2.1. Study population

The WHI-OS is a longitudinal cohort of 93,676 postmenopausal women aged 50–79 years who were recruited at 40 different clinical centers across the United States between October 1, 1993 and December 31, 1998 [32]. The present study was based on a case-cohort study population used for previous studies of CRC within the WHI-OS [33,34]. A case of incident CRC was defined and adjudicated according to International Classification of Diseases for Oncology site codes 153.0–153.4, 153.6–153.9, and 154.0–154.1. Study subjects were excluded if they were diagnosed with CRC or censored in the first year of follow-up; had taken diabetes treatment at baseline; or had a history of cancer of colon-rectum, breast, or endometrium. We identified 496 women who developed primary CRC with follow-up through February 29, 2004. A subcohort of 892 women was randomly selected from women who did not develop cancer as of February 29, 2014 [33]. The study protocol was approved by Institutional Review Board of each participating clinical center of the WHI, Baylor College of Medicine, and Michael E. DeBakey VA Medical Center (MED-VAMC).

2.2. Data and bio-specimen collection at baseline

At enrollment, women in the WHI-OS provided informed consent and completed questionnaires regarding demographic and lifestyle/behavioral factors, family history of

cancer and medication use. They also completed a validated food frequency questionnaire on food consumption during the prior three months from which daily average nutrient consumption was computed as described elsewhere [35]. Trained WHI staff measured waist and hip circumference, height, and weight. BMI (weight (kg)/height (m)²) and waist to hip ratio (WHR) variables were calculated. At baseline, blood samples were obtained following an overnight fast of at least 12 h and stored at -70°C. Because the plasma/serum samples have been used to test biomarkers previously, all samples have gone through one freeze-thaw cycle, except for one plasma sample that went through two freeze-thaw cycles.

2.3. Measurement of circulating levels of CML-AGE, sRAGE, and other biomarkers

Citrate-plasma was used for measuring CML-AGE using a commercially available ELISA kit (Microcoat Biotechnologie Company, Bernried, Germany). Serum or EDTA-plasma was used for measuring sRAGE using human sRAGE Quantikine ELISA kit (R&D System Inc., Minneapolis, MN). Randomly ordered case and subcohort sample were run together in each batch (96-well plate). A 10% quality control (QC) sample provided by the WHI clinical coordinating center was also randomly placed on each plate. The laboratory personnel who performed the ELISA assay were blinded to QC and case-subcohort status. All samples were assayed in duplicate by the same personnel using kits with the same lot number.

Among 496 cases and 892 subcohort members, we included 442 cases and 800 subcohort members for CML-AGE testing and 318 cases and 638 subcohort members for sRAGE testing due to the use of residual samples from other studies. We included 396 cases and 706 subcohort members in the analysis of CML-AGE after excluding unreliable measurement points as determined by intraplate coefficient of variation (CV) >12%. Data on insulin, adipokines (adiponectin and leptin) and CRP were available for 457 cases and 841 subcohort member as part of earlier studies [33,34].

2.4. Statistical analysis

Untransformed CML-AGE and sRAGE levels were summarized by presenting their median values and interquartile ranges. The reproducibility of the lab assay was evaluated using intra-plate and inter-plate CV, and interclass correlation coefficient (ICC) based on the data of the QC samples. We examined the distribution of multiple factors across quartiles of CML-AGE and sRAGE using the Wilcoxon rank sum test or the ANOVA test for continuous variables and Pearson's χ^2 test for categorical variables. We examined the correlation between CML-AGE and sRAGE and exposure variables using non-parametric Spearman's rank correlation analysis.

In this case-cohort study, the survival time was censored at the time of diagnosis for CRC, date of death, or February 29, 2004, whichever came first. We used Cox proportional hazards regression model to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) for the association of incident CRC with CML-AGE or sRAGE. We calculated biomarker quartile cut-points according to their distributions in the subcohort, and assessed risk estimates for trend using a Wald test by treating the categories as the ordinal variable. All Cox models were computed with robust variance estimation in order to account for the case-cohort design [36,37]. We tested whether all models met the proportional hazard

assumption using a published method [38]. Non-linearity using sRAGE as a continuous variable in predicting CRC risk was tested by restricted cubic spline regression [39].

Confounding factors were those associated with both CRC and CML-AGE or sRAGE (P value <0.25 in univariate analysis) or if their inclusion changed the risk estimates by more than 10%, which included age, physical activity (metabolic equivalent of task (MET)-hour/week), energy intake, alcohol use (serving per week), consumptions of saturated fat, available carbohydrate, calcium, total folate, red meat, and waist circumference as continuous variables; and race/ethnicity, smoking status and hormone replacement therapy (categorized as never, former, and current), family history of colorectal cancer, self-reported type 2 diabetes, hypertension, and use of NSAIDs (yes *versus* no) as categorical variables. All adiposity measures were individually associated with both CRC and sRAGE and CML-AGE. However, given multi-collinearity we were unable to include all three. We therefore conservatively included waist circumference in the multivariate model because this variable attenuated the sRAGE risk estimate the most compared to BMI and WHR. Although we excluded women with self-reported treated type 2 diabetes, there were 20 women with self-reported untreated type 2 diabetes. This variable (yes *versus* no) was included in the model. Freeze-thaw cycle of the samples was evaluated as an additional confounding factor but did not change the risk estimate. Furthermore, certain medications including statins have been shown to increase sRAGE levels by increasing RAGE shedding in animal models [40] as well as in human [41]. However, in this study, we did not find statin use (yes *versus* no) confounded the association between sRAGE and CRC. Diet and food variables were energy-adjusted using the density method. Levels of sRAGE (for CML-AGE) or CML-AGE (for sRAGE), insulin, adiponectin, and CRP were included additionally. We draw final conclusions based on the fully adjusted HRs.

We performed stratified analyses by three *a priori* variables: baseline BMI (<25 *versus* ≥ 25 kg/m²), insulin and CRP (median among subcohort as cut-off point). The statistical significance of the interactions was tested using a Wald test on the cross-product interaction term introduced into the multivariable Cox model. To further examine the relation between CML-AGE or sRAGE and insulin, we examined whether adjustment for CML-AGE or sRAGE changed the association between insulin and risk of CRC, and *vice versa*. Because sRAGE levels were higher when serum samples were used as the testing material compared with when EDTA-plasma samples were used (1630 pg/ml among 410 subcohort members using serum *versus* 1505 pg/ml among 228 subcohort members using EDTA-plasma, $P=0.02$), in a sensitivity analysis, we also generated sample-type specific quartiles to estimate the risk. We also excluded data generated using the EDTA-plasma to examine the association between sRAGE and risk of pancreatic cancer. In addition, we performed sensitivity analyses in those who had been followed up for no less than five year in risk association analysis for sRAGE. Finally, because of the potential biological interaction between CML-AGE and sRAGE, we generated the mutually adjusted CML-AGE or sRAGE value using the residual method [42] and we performed sensitivity analyses by using these mutually adjusted values as the exposure variables for risk estimates.

All statistical analyses were performed using SAS 9.1 (SAS institute Inc., Cary, NC). All tests were two-sided with P -values < 0.05 indicative of statistical significance.

3. Results

3.1. Baseline characteristics and QC data

General characteristics of the cases *versus* non-cases were previously reported [33,34] (Supplemental Table 1). The HR was 1.49 (95% CI: 1.08–2.08) for BMI ≥ 25 kg/m² compared with BMI < 25 kg/m² after adjusting for age, race, smoking status, energy intake, physical activity, alcohol intake and family history of cancer. The baseline level of CML-AGE was significantly lower in cases than in the non-cases ($P = 0.04$). Table 1 shows general characteristics of the subcohort according to quartile (Q) of CML-AGE or sRAGE. Women with higher CML-AGE or sRAGE levels were more likely to be non-Hispanic white. Both CML-AGE and sRAGE were significantly inversely associated with BMI, waist circumference, WHR, and insulin levels, while positively associated with adiponectin. CML-AGE was positively associated with sRAGE and total fiber intake, but was inversely associated with red meat intake. sRAGE was positively associated with NSAIDs use and calcium intake, but inversely associated with hypertension, alcohol consumption, and CRP level (P values < 0.05). Similarly, among the subcohort members, CML-AGE and sRAGE were inversely correlated with BMI, insulin and CRP, but positively correlated with adiponectin (P values < 0.0001) (Table 2).

Using the blinded QC data (28 pairs for sRAGE and 26 pairs for CML-AGE), we calculated the inter-plate CV and ICC for both markers. The interplate CV was 7.85% for sRAGE and was 14.8% for CML-AGE. The ICC was 94.5% for sRAGE and 67.0% for CML-AGE.

3.2. Main effect

In univariate analyses, post-menopausal women with higher levels of CML-AGE were significantly less likely to develop CRC, although the significant association was not found in multivariable analyses (Table 3). For sRAGE, women in the 2nd quartile of baseline levels of sRAGE had significantly reduced CRC risk (HR_{adjQ2} *versus* Q1 = 0.52, 95% CI: 0.30–0.92) in multivariate models, although a dose-response pattern for the association was not observed (P for trend = 0.90). However, the test of non-linearity of continuous sRAGE in predicting risk of CRC via spline regression did not reject the linearity hypothesis ($P = 0.18$).

3.3. Effect modification and sensitivity analysis

Table 4 shows that there were no significant interactions of CML-AGE by BMI, insulin and CRP levels. Table 5 shows the results for stratified analysis of sRAGE and CRC risk according to BMI, insulin, and CRP. There was a significant inverse association between the highest levels of sRAGE and CRC among women with BMI ≥ 25 kg/m², but this inverse association was not seen among women with BMI < 25 kg/m² (P value for interaction 0.01). The interaction with insulin and CRP was not statistically significant.

The sensitivity analyses using the mutually adjusted values of CML-AGE and sRAGE for modeling (Supplemental Table 2) showed similar findings to the main analyses. An interaction effect by BMI was also observed (Supplemental Table 3). Using the sample-type specific cutoff points for sRAGE, we observed the same trend of the association between

sRAGE and pancreatic cancer risk. The multivariable HR was 0.64 (95% CI: 0.38–1.09), 0.81 (95% CI: 0.47–1.40), and 0.81 (0.46–1.41) for the second, third, and fourth quartile, respectively, compared with the first quartile ($P_{\text{trend}} = 0.59$) (Model 5 in Table 2). After excluding data from 205 cases and 410 subcohort members with EDTA plasma determined sRAGE, the HRs were 0.99 (95% CI 0.52–1.02), 0.85 (95% CI: 0.42–1.70), and 0.85 (95% CI: 0.43–1.71) for the second, third, and fourth quartile compared with the first quartile ($P_{\text{trend}} = 0.58$), respectively. In addition, the interaction effect of sRAGE* BMI in our sensitivity analysis was similar to the main analysis. Among the study subjects with BMI ≥ 25 kg/m², the HR for CRC was 0.96 (95% CI: 0.40–2.30), 0.90 (95% CI: 0.34–2.38), and 0.43 (0.14–1.32) for the second, third, and fourth quartile, respectively, compared with the first quartile of sRAGE ($P_{\text{trend}} = 0.19$). When we limited the analysis among those who were followed up for ≥ 5 years (median follow-up 6.2 years), we found a stronger association between sRAGE and CRC. The HR was 0.51 (95% CI 0.18–1.43), 1.36 (95% CI: 0.52–3.59), and 0.50 (95% CI: 0.15–1.58) for the second, third and fourth quartile of sRAGE compared with the lowest quartile.

Finally, we found that higher levels of insulin were associated with significantly increased CRC risk in our cohort (HR_{adjQ4 versus Q1}: 1.64 (95% CI: 1.09–2.48)). Further adjustment for either CML-AGE or sRAGE changed the HRs by less than 10% (e.g., HR_{adjQ4 versus Q1}: 1.56, 95% CI: 1.02–2.38 for CML-AGE adjustment and HR_{adjQ4 versus Q1}: 1.53, 95% CI: 0.94–2.50 for sRAGE adjustment, respectively).

4. Discussion

In this prospective case-cohort study of postmenopausal women, we did not observe a significant association between baseline circulating CML-AGE and sRAGE levels and CRC risk. We found an inverse association between pre-diagnostic sRAGE levels and the subsequent risk of incident CRC in women with BMI ≥ 25 kg/m², independent of insulin levels and CRP. There was no inverse association between sRAGE and CRC in women with normal BMI.

sRAGE is a decoy receptor for RAGE ligands, including CML-AGE, pro-inflammatory high mobility group Box 1 (HMGB1) and S100 family proteins, all of which have been implicated in colorectal carcinogenesis [43,44]. In animal models, one study found that high-fat feeding induced expression of two RAGE ligands, HMGB1 and CML-AGE, in the liver and adipose tissue. Deficiency of RAGE or administration of sRAGE partially blocked the high-fat-diet induced inflammation and weight gain [45]. Circulating levels of sRAGE have been inversely associated with CRP levels and BMI [27,28]. Our previous studies on sRAGE in men found a significant inverse association with the risk of developing CRC [29] and colorectal adenomas [30]. Our present findings were in line with these observations and implied that higher circulating levels of sRAGE may confer a stronger protective effect against CRC in women with BMI ≥ 25 kg/m². The crosstalk between RAGE and adiposity has been demonstrated in progression of atherosclerosis in an apoE/RAGE knock-out mouse study [46]. Endogenous secretory RAGE has been associated with platelet activation and oxidative stress among obese women [47]. Platelet count and activation have also been implicated in CRC development [48]. Further understanding of the RAGE-ligand interaction

in the tissue microenvironment, as well as the production of sRAGE under inflammatory or obese conditions, may aid interpretation of our study findings.

Our study found circulating CML-AGE was positively correlated with adiponectin, and inversely correlated with indices of excessive adiposity and insulin. This finding was consistent with other studies conducted in children or healthy adults [49,50], but was in contrast to the reported positive association between CML-AGE and insulin resistance and chronic inflammatory diseases [51,52] including incident cardiovascular risk both in type 2 diabetics [53] and in healthy older adults [54]. It is unknown whether this discrepancy was due to different study populations in terms of age, sex, or chronic disease status. Several recent studies examined the involvement of the AGEs/RAGE axis in adiposity [46,55,56]. An animal study showed that CML-AGE accumulation in conjunction with increased expression of RAGE in adipose tissue contributes to the dysregulation of adipokines in obesity and insulin resistance [16]. RAGE-mediated trapping of CML-AGE in the adipose tissue may explain decreased plasma levels of CML in obese subjects [16] and hence our study finding. However, we did not observe a significant interaction between CML-AGE and BMI on CRC risk in our study. Because levels of CML-AGE have also been shown to be modulated by its receptors, including galectin 3, macrophage scavenger receptor [57], AGER-1 and sirtuin 1 [58], its full impact at the tissue level may need to be considered in the context of its receptors.

We observed an overall trend of an inverse association between sRAGE and CRC which was also observed for CRC in the ATBC Finnish male smoker cohorts [29]. However, some discrepancies were also noted. For example, we did not find an association between CML-AGE and CRC in the present study in contrast to the ATBC Study. The source of AGEs was different in these two cohorts in that the study subjects in the ATBC Study might have been exposed to both tobacco AGEs and dietary AGEs. In the WHI, only 6% of the women were current smokers and diet may have been the major source of AGEs. However, compared with the ATBC Study, women in the WHI cohort had slightly higher levels of CML-AGE (561 ng/ml in ATBC *versus* 586 ng/ml in WHI, median), and much higher levels of sRAGE (572 pg/ml in ATBC *versus* 1463 pg/ml in WHI, median) although the same type of ELISA assay kits were used in two studies. Due to the observed higher levels of sRAGE in women than men, it is unknown whether the harmful effect of CML-AGE was offset by higher sRAGE in the WHI Study. In addition, unlike the present study, there was no interaction between sRAGE and BMI in the ATBC Study. While nearly 70% had a BMI greater than 25 kg/m² in the WHI Study, the Finnish male smokers were generally lean. The narrow and lean range of BMI in the ATBC Study might explain the absent interaction between CML-AGE/sRAGE and BMI. Collectively, our observations indicated that the effect of CML-AGE and sRAGE on CRC risk may depend on exposure characteristics such as BMI and smoking. However, additional research is needed to further elucidate potential gender differences (such as sex hormones) in these associations.

Our study had several limitations. First, we used previously thawed biological samples from prior case-cohort studies. Nevertheless, the study samples went through the same freeze-thaw cycles. Moreover, the adjustment for freeze-thaw cycle did not materially change risk estimates. For CML-AGE, the steps of sample dilution and proteinase K digestion

introduced variability resulting in the need to exclude data from 140 participants. However, the characteristics of these 140 participants were not different from the rest of the study subjects used in data analysis. We used both serum and EDTA plasma in measuring sRAGE. Measurement errors due to the sample resource could have compromised the accuracy of the data. However, the sensitivity analysis showed the same trend of association as the main finding we reported. Second, the association between sRAGE levels and CRC risk was not linear, which complicates the potential dose-response based support of causality or effect mediation. Research in additional cohorts is necessary to help further refine understanding of biological interaction between sRAGE and the host system. For example, sRAGE levels could have been affected by polymorphisms of *RAGE* (*AGER*) [59,60]. Genetic background should be considered in future studies. Finally, we performed multiple stratified analyses to explore the plausible association between CML-AGE/sRAGE, adiposity, insulin and CRP. We did not exclude the possibility of a false positive finding based on the limited sample size. The observed interaction effect between sRAGE and BMI needs to be interpreted with caution especially in the absence of dose-response seen for sRAGE among women with higher BMI.

5. Conclusions

We report a novel finding suggesting the involvement of sRAGE in obesity-related CRC in post-menopausal women. The balance between the synthesis of anti-inflammatory sRAGE and pro-inflammatory RAGE ligands may be an important determinant of RAGE mediated reactions. Further studies with larger sample size are needed to elucidate the inter-related mechanisms among adiposity, insulin resistance, chronic inflammation, and RAGE-ligand axis in diverse populations. Broadly, our research may have implications concerning the role of pattern recognition receptors such as RAGE in fostering an inflammatory tumor microenvironment in the development of CRC.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Baseline characteristic of study subjects according to levels of CML-AGE or sRAGE in the subcohort.

Table 1

Characteristics ^d (mean (SD) or %)	CML-AGE ^b (quartile) n=561				P value	sRAGE ^b (quartile) n=638				P value
	1	2	3	4		1	2	3	4	
Range	185–463	464–585	586–706	707–1339		346–1130	1131–1461	1465–1963	1964–5043	
Age (year)	62.0 (7.1)	62.0 (7.4)	63.3 (7.1)	63.4 (7.8)	0.10	63.2 (7.0)	62.6 (7.7)	62.9 (7.5)	62.2 (7.4)	0.70
Race, the other line % non-Hispanic White	81.2	80.8	88.2	90.3	0.03	76.9	86.2	88.1	93.7	<0.0001
% African American	8.52	9.60	1.69	5.71		14.9	4.00	1.25	0.63	
Body mass index, % of 25	71.9	63.0	59.2	48.5	0.0002	67.8	62.7	54.2	51.0	0.04
Waist to hip ratio	0.82 (0.07)	0.80 (0.08)	0.79 (0.07)	0.78 (0.07)	<0.0001	0.82 (0.07)	0.81 (0.07)	0.79 (0.07)	0.78 (0.06)	<0.0001
Waist circumference (cm)	89.8 (14.6)	84.8 (12.8)	82.3 (10.5)	80.5 (11.5)	<0.0001	88.2 (14.2)	86.0 (13.1)	82.0 (11.4)	80.4 (11.0)	<0.0001
Physical activity (MET-hour/wk)	11.7 (12.3)	14.1 (14.9)	15.0 (16.9)	14.6 (13.8)	0.14	12.8 (15.4)	13.3 (14.8)	16.1 (14.2)	14.2 (15.0)	0.21
Smoking status					0.89					0.08
Never smokers, %	50.3	57.5	55.1	54.0		44.0	52.5	60.1	58.2	
Former smokers, %	41.1	36.2	38.6	39.1		48.4	43.1	36.1	36.1	
Current smokers, %	8.6	6.3	6.3	6.9		7.6	4.4	3.8	5.7	
20 years of smoking, %	61.9	50.7	45.4	53.2	0.15	60.5	49.3	45.0	46.7	0.12
Colitis, % yes	1.14	2.31	0.56	0.57	0.37	0.63	1.27	0.64	2.55	0.38
Received colonoscopy, % yes	53.1	52.3	56.2	58.6	0.62	56.9	53.2	56.0	59.9	0.69
Hypertension, % yes	30.5	34.9	25.8	28.0	0.29	36.1	34.2	27.0	22.0	0.02
Self-reported diabetes, % yes	2.3	2.3	0.6	1.1	0.47	4.4	0.6	1.3	1.3	0.06
Family history of CRC, % yes	17.4	16.7	14.9	18.4	0.86	18.4	11.0	13.9	21.2	0.08
Hormone replacement therapy					0.72					0.16
Never users, %	33.8	38.0	38.8	45.9		41.5	40.3	37.6	36.0	
Former users, %	15.0	17.1	15.6	14.5		16.5	14.8	18.5	17.1	
Current users, %	51.2	44.9	45.6	39.6		42.0	44.9	43.8	46.9	
NSAIDs use once per week	14.8	21.5	14.0	18.3	0.22	18.1	12.0	15.6	23.9	0.04

Characteristics ^a (mean (SD) or %)	CML-AGE ^b (quartile) n=561				sRAGE ^b (quartile) n=638			
	1	2	3	4	1	2	3	4
Total energy intake (Kcal)	1630 (663)	1499 (582)	1497 (570)	1519 (563)	1565 (628)	1571 (600)	1617 (613)	1513 (611)
Protein (g)	30.8 (18.9)	34.0 (19.6)	33.7 (20.7)	32.3 (14.6)	32.3 (16.2)	30.8 (17.9)	30.5 (14.8)	32.4 (16.0)
Total fat (g)	25.3 (12.8)	25.9 (15.2)	26.0 (16.5)	23.0 (11.1)	24.7 (14.8)	24.9 (12.0)	23.6 (12.3)	23.9 (12.7)
Saturated fat (g)	8.35 (4.23)	8.41 (5.19)	8.54 (5.54)	7.60 (3.76)	8.11 (5.08)	8.12 (3.99)	7.76 (3.93)	8.05 (4.67)
Red meat (servings)	0.32 (0.26)	0.28 (0.26)	0.29 (0.29)	0.24 (0.20)	0.29 (0.26)	0.28 (0.23)	0.26 (0.22)	0.25 (0.22)
Available carbohydrate (g)	84.6 (42.9)	94.2 (56.9)	97.5 (57.8)	96.4 (47.5)	88.4 (42.1)	88.3 (43.3)	89.8 (50.8)	99.1 (49.7)
Total fiber (g)	7.43 (4.96)	9.02 (8.14)	8.91 (6.30)	9.18 (5.74)	8.09 (5.27)	8.25 (5.50)	8.07 (5.08)	8.93 (5.64)
Folate equivalent (µg)	225 (149)	268 (215)	256 (166)	260 (155)	234 (126)	237 (156)	245 (195)	268 (154)
Calcium (mg)	506 (201)	527 (208)	532 (227)	565 (216)	524 (224)	499 (193)	563 (212)	574 (210)
Alcohol (servings per week)	2.49 (4.84)	2.80 (6.68)	2.49 (4.48)	2.91 (6.39)	3.42 (7.2)	3.63 (6.9)	2.25 (3.5)	1.82 (3.6)
CML-AGE (ng/ml)					564 (181)	589 (175)	627 (187)	637 (184)
sRAGE (pg/ml)	1377 (566)	1602 (595)	1587 (624)	1702 (689)	<0.0001			
Insulin (uIU/ml)	8.28 (5.50)	6.87 (4.94)	6.24 (4.18)	5.84 (4.68)	7.97 (5.7)	7.54 (5.1)	5.94 (4.3)	5.46 (3.9)
Adiponectin (µg/ml)	26.7 (12.5)	29.6 (14.3)	31.2 (15.4)	32.1 (14.0)	27.5 (14.4)	29.8 (14.9)	30.2 (12.9)	33.3 (14.5)
Leptin (ng/ml)	21.3 (16.4)	19.4 (29.8)	17.7 (13.9)	17.2 (17.7)	22.2 (32.7)	19.9 (17.5)	15.9 (12.5)	17.6 (17.4)
C-reactive protein (mg/l)	4.24 (6.89)	3.28 (4.20)	3.11 (4.08)	3.13 (4.26)	4.05 (4.93)	4.22 (7.42)	3.37 (4.17)	2.47 (2.51)

AIDs, Nonsteroidal Anti-inflammatory Drugs.

^aFood variables were energy-adjusted.

Table 2

Spearman correlation coefficient (r) for the selected characteristics with CML-AGE and sRAGE in the subcohort in the WHI-OS Study [1993–2004].

Characteristics	CML-AGE	sRAGE
	r	r
Age at randomization	0.09 ^a	−0.05
BMI, kg/m ²	−0.22 ^b	−0.23 ^b
Waist circumference (cm)	−0.23 ^b	−0.23 ^b
Waist to hip ratio	−0.22 ^b	−0.22 ^b
Years of smoking	0.06	−0.13 ^a
Total fat (g/day)	−0.12 ^a	−0.0007
Saturated fat (g/day)	−0.11 ^a	0.003
Available carbohydrate intake (g/day)	0.08 ^a	0.09 ^a
Total sugar (g/day)	0.11 ^a	0.08 ^a
Protein (g/day)	0.02	0.03
Red meat (g/day)	−0.15 ^a	−0.04
Processed meat (g/day)	−0.15 ^a	−0.01
Glucose intake (g/day)	0.12 ^a	0.07
Fructose intake (g/day)	0.12 ^a	0.06
Alcohol consumption	0.08	−0.06
Calcium (g/day)	0.10 ^a	0.11 ^a
Serum insulin	−0.19 ^b	−0.23 ^b
Serum glucose	−0.12 ^a	−0.21 ^b
Serum IGF-1	−0.0005	0.01
Serum adiponectin	0.17 ^a	0.20 ^b
Serum leptin	−0.11 ^a	−0.11 ^a
CRP	−0.15 ^a	−0.097 ^a
sRAGE	0.16 ^a	1.0 0

^a $P < 0.05$.

^b $P < 0.0001$.

Table 3

Hazard ratio (HR) and 95% confidence interval (CI) of colorectal cancer by quartile of CML-AGE or sRAGE levels in the WHI-OS [1993–2004].

Biomarker	Quartile				P for trend
	1	2	3	4	
CML-AGE (ng/ml, range)	185–463	464–585	586–706	707–1339	
Case/non-cases (n/n)	116/176	108/177	84/17	87/175	
HR 95% CI ^a	1.00	0.92	0.65–1.31	0.46–0.96	0.69 0.48–1.00 0.02
HR 95% CI ^b	1.00	1.00	0.67–1.51	0.53–1.24	0.81 0.52–1.26 0.24
HR 95% CI ^c	1.00	1.07	0.67–1.75	0.40–1.15	0.81 0.48–1.38 0.22
HR 95% CI ^d	1.00	0.99	0.60–1.63	0.37–1.10	0.81 0.48–1.37 0.24
HR 95% CI ^e	1.00	1.11	0.68–1.82	0.41–1.19	0.78 0.46–1.34 0.18
HR 95% CI ^f	1.00	0.87	0.62–1.76	0.40–1.22	0.85 0.49–1.47 0.34
sRAGE (pg/ml, range)	346–113 0	1125–1461	1465–1963	1964–5043	
Case/non-cases (n/n)	95/160	66/159	87/160	69/159	
HR 95% CI ^a	1.00	0.71	0.47–1.05	0.64–1.35	0.74 0.50–1.10 0.30
HR 95% CI ^b	1.00	0.71	0.44–1.14	0.72–1.78	0.83 0.50–1.37 0.86
HR 95% CI ^c	1.00	0.59	0.35–1.01	0.68–1.86	0.87 0.51–1.51 0.88
HR 95% CI ^d	1.00	0.54	0.31–0.93	0.62–1.78	0.83 0.47–1.46 0.98
HR 95% CI ^e	1.00	0.55	0.31–0.96	0.61–1.79	0.84 0.48–1.48 0.98
HR 95% CI ^f	1.00	0.52	0.30–0.92	0.56–1.69	0.81 0.45–1.45 0.90

^aModel 1: Adjusted for age.

^bModel 2: Model 1 + physical activity, waist circumference, alcohol consumption, energy intake, consumption of saturated fat, available carbohydrate, calcium, folate equivalent, and red meat (continuous), and race, smoking, family history of colorectal cancer, NASIDs use, hypertension, diabetes, and hormone replacement therapy (categorical).

^cModel 3: Model 2 + sRAGE (continuous) for CML-AGE, or CML-AGE (continuous) for sRAGE.

^dModel 4: Model 3 + adiponectin (continuous).

^eModel 5: Model 4 + insulin (continuous).

^fModel 6: Model 5 + CRP (continuous).

Table 4

Association between CML-AGE and risk of incident CRC by BMI, insulin, and CRP in the WHI-OS [1993–2004].

Factors	Cases/Non-cases	HR ^a	95% CI ^a	HR ^b	95% CI ^b	Cases/Non-cases	HR ^a	95% CI ^a	HR ^b	95% CI ^b
BMI <25 kg/m ²						BMI ≥25 kg/m ²				
Q1	33/53	1.00		1.00	1.1.1	83/123	1.00	1.1.2	1.00	1.1.3
Q2	31/68	0.68	0.25–1.82	0.52	0.18–1.55	77/109	1.18	0.60–2.34	1.25	0.63–2.48
Q3	39/74	0.40	0.14–1.10	0.30	0.10–0.93	45/104	0.69	0.32–1.53	0.78	0.35–1.76
Q4	37/92	0.44	0.17–1.16	0.33	0.11–1.00	50/83	0.89	0.43–1.87	1.02	0.49–2.13
P for trend		0.06		0.04			0.50		0.77	
P for interaction	0.49									
Insulin < median						Insulin ≥ median				
Q1	39/64	1.00	1.1.4	1.00		77/112	1.00	1.1.5	1.00	1.1.6
Q2	42/80	0.87	0.37–2.04	0.77	0.30–1.93	66/97	1.17	0.58–2.37	1.23	0.60–2.50
Q3	37/95	0.45	0.19–1.10	0.39	0.15–1.01	47/83	0.79	0.38–1.65	0.85	0.40–1.79
Q4	48/110	0.69	0.28–1.72	0.62	0.23–1.71	39/65	0.70	0.30–1.61	0.77	0.33–1.78
P for trend		0.26		0.24			0.29		0.40	
P for interaction	0.89									
CRP < median						CRP ≥ median				
Q1	46/82	1.00		1.00		70/94	1.00		1.00	
Q2	47/93	0.69	0.32–1.49	0.70	0.30–1.65	61/84	1.48	0.73–2.97	1.41	0.70–2.85
Q3	47/95	0.66	0.30–1.44	0.69	0.29–1.62	37/83	0.61	0.26–1.41	0.58	0.24–1.41
Q4	41/104	0.82	0.39–1.72	0.97	0.43–2.18	46/71	0.63	0.25–1.61	0.69	0.27–1.77
P for trend		0.61		0.995			0.82		0.20	
P for interaction	0.15									

^aModel 1: Adjusted for age, physical activity, alcohol consumption, energy intake, consumption of saturated fat, available carbohydrate, calcium, folate equivalent and red meat, sRAGE, adiponectin, and insulin (continuous), and race, waist circumference, smoking, family history of colorectal cancer, NASIDs use, hypertension, diabetes, hormone replacement therapy (categorical).^bModel 2: Model 1 + CRP (continuous).

Table 5

Association between sRAGE and incident CRC by BMI, insulin and CRP in the WHI-OS [1993–2004].

Factors	Cases/Non-cases	HR ^a	95% CI ^a	HR ^b	95% CI ^b	Cases/Non-cases	HR ^a	95% CI ^a	HR ^b	95% CI ^b
BMI <25 kg/m ²						BMI ≥25 kg/m ²				
Q1	23/57	1.00		1.00	1.1.7	72/103	1.00	1.1.8	1.00	1.1.9
Q2	17/60	0.60	0.20–1.77	0.56	0.17–1.87	49/99	0.49	0.24–1.01	0.49	0.24–1.01
Q3	30/76	0.98	0.34–2.86	1.02	0.31–3.31	57/84	1.08	0.55–2.11	0.95	0.48–1.88
Q4	40/79	1.88	0.65–5.40	2.39	0.69–8.24	29/80	0.42	0.18–0.97	0.39	0.17–0.91
P for trend		0.17		0.13			0.19		0.11	
P for interaction	0.01									
Insulin < median						Insulin ≥ median				
Q1	26/64	1.00	1.1.10	1.00	1.1.11	69/96	1.00	1.1.12	1.00	1.1.13
Q2	29/70	0.48	0.17–1.32	0.39	0.13–1.14	37/89	0.59	0.28–1.27	0.56	0.26–1.22
Q3	42/92	1.07	0.44–2.63	0.89	0.32–2.45	45/68	0.97	0.46–2.06	0.93	0.44–1.97
Q4	40/95	1.15	0.48–2.80	1.00	0.38–2.66	29/64	0.59	0.25–1.44	0.58	0.24–1.43
P for trend		0.37		0.52			0.40		0.38	
P for interaction	0.06									
CRP < median						CRP ≥ median				
Q1	34/70	1.00		1.00		61/90	1.00		1.00	
Q2	23/83	0.41	0.15–1.11	0.32	0.10–0.95	43/76	0.63	0.27–1.50	0.67	0.28–1.61
Q3	46/82	1.24	0.56–2.73	1.08	0.44–2.62	41/78	0.86	0.39–1.94	0.88	0.39–1.99
Q4	39/99	0.94	0.43–2.07	0.87	0.36–2.08	30/60	0.50	0.17–1.53	0.47	0.15–1.46
P for trend		0.49		0.59			0.28		0.25	
P for interaction	0.21									

^aModel 1: Adjusted for age, physical activity, alcohol consumption, energy intake, consumption of saturated fat, available carbohydrate, calcium, folate equivalent and red meat, CML-AGE, adiponectin, and insulin (continuous), and race, waist circumference, smoking, family history of colorectal cancer, NASIDs use, hypertension, diabetes, hormone replacement therapy (categorical).

^bModel 2: Model 1 + CRP (continuous).