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Advanced Glycation End-Products, Soluble Receptor for Advanced Glycation End-Products and Risk of Colorectal Cancer

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Abstract

Background—Advanced glycation end-products (AGEs) accumulate in human tissue proteins during aging, particularly under hyperglycemia conditions. AGEs induce oxidative stress and inflammation via the receptor for AGEs (RAGE) and soluble RAGE (sRAGE) can neutralize the effects mediated by RAGE/ligand engagement.

Methods—We examined the association between N^{ε}-(carboxymethyl)lysine (CML), a prominent AGEs, and sRAGE and colorectal cancer risk in a prospective case-cohort study nested within a cancer prevention trial among 29,133 Finnish male smokers. Among study subjects who were alive without cancer five years after baseline (1985–1988), we identified 483 incident colorectal cancer cases and randomly sampled 485 subcohort participants as the comparison group with the follow-up to April 2006. Baseline serum levels of CML-AGE, sRAGE, glucose and insulin were determined. Weighted Cox proportional hazard regression models were used to calculate relative risks (RR) and 95% confidence intervals (CI).

Results—Comparing highest with lowest quintile of sRAGE, the RR for incident colorectal cancer was 0.65 (95% CI: 0.39, 1.07; *P* value for trend = 0.03), adjusting for age, years of smoking, body mass index, and CML-AGE. Further adjustment for serum glucose strengthened the association (RR: 0.52; 95% CI: 0.30, 0.89; *P* value for trend = 0.009). Highest quintile of CML-AGE was not associated with an increased risk of colorectal cancer (multivariate RR: 1.20; 95% CI: 0.64, 2.26).

Conclusion—Higher prediagnostic levels of serum sRAGE were associated with lower risk of colorectal cancer in male smokers.

Impact—This is the first epidemiologic study to implicate the receptor for advanced glycation end-products in colorectal cancer development.

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Keywords

advanced glycation end-products; soluble receptor for advanced glycation end-products; colorectal cancer; risk; case-cohort; inflammation

Introduction

Energy imbalance, insulin resistance, and chronic inflammation are underlying mechanisms in colorectal cancer development. Further understanding of interrelations among environmental exposure (such as dietary intake and smoking) and these etiological mechanisms may provide novel opportunities for prevention and clinical management of colorectal cancer. We proposed that the axis of advanced glycation end-products (AGEs) and receptor for AGEs (RAGE) contributes to the development of colorectal cancer.

AGEs are a group of irreversible adducts or crosslinking created through a nonenzymatic glycosylation between reducing sugars and free amino groups of proteins, lipids, or nucleic acids (1). AGEs form endogenously during normal metabolism, and exogenously from foods processed at high temperatures, as well as from tobacco smoking (2;3). AGEs accumulate slowly in human tissue proteins during aging and more rapidly in the states associated with hyperglycemia and enhanced oxidative and carbonyl stress. Circulating indicators of AGEs and oxidative stress are directly influenced by the intake of dietary AGEs (4).

The principal mechanism by which AGEs elicit biological function is through their receptors. The ligation of AGEs and membrane-bound full-length RAGE can trigger an array of signaling pathways that are involved in inflammation and tumorigenesis (5). Soluble form of RAGE (sRAGE) is found in the circulation in humans (6). By binding AGEs or other ligands and acting as a “receptor decoy”, sRAGE represents a naturally occurring competitive inhibitor of RAGE-mediated signaling pathways (7).

The AGEs-RAGE axis plays a critical role in the pathological interplay between hyperglycemia and vascular homeostasis. However, the role of AGEs in cancer development is largely unknown. Several hospital-based studies found decreased sRAGE levels in patients with breast cancer, lung cancer, and pancreatic cancer, compared to healthy controls (8–10). In the present study, we prospectively investigated the associations of N^{ε}-(carboxymethyl)lysine (CML), a prominent type of AGEs, and sRAGE with risk of colorectal cancer in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study (11). We hypothesized that higher levels of CML-AGE and lower levels of sRAGE are associated with a greater risk of colorectal cancer.

Materials and Methods

Study design and participants

The ATBC Study is a primary prevention trial conducted in southwest Finland. Between 1985 and 1988, 29,133 men aged 50 to 69 who smoked at least five cigarettes per day were randomized to receive α-tocopherol, β-carotene, α-tocopherol and β-carotene, or placebo. Exclusion criteria for the participation in the trial included: malignancy other than non-melanoma skin cancer or carcinoma in situ; severe angina on exertion; chronic renal insufficiency; cirrhosis of liver; chronic alcoholism; receiving anticoagulant therapy; other medical problems that might limit participation for six years; and current use of supplements containing vitamin E, vitamin A or beta-carotene (11). The trial ended in April 1993 and the follow-up for health outcomes continues through national registries. The ATBC Study was approved by the institutional review groups of both US National Cancer Institute and the

National Public Health Institute of Finland (now National Institute for Health and Welfare, Helsinki, Finland). All participants provided the written informed consent before randomization.

The present study is a case-cohort study within the ATBC Study. Eligible study subjects were alive without cancer within the first five years of follow-up after baseline (N = 24,708). The follow-up ended at death, diagnosis of colorectal cancer, or on April 30, 2006. A total of 508 incident cases of colorectal cancer were identified from the Finnish Cancer Registry (12), including proximal tumors (ICD-9 codes 153.0, 153.1, 153.4, 153.6, 153.7) and distal tumors (ICD-9 codes 153.2, 153.3, 154.0, 154.1). A reference group was comprised of five hundred subcohort participants who were randomly selected from all eligible study subjects. After excluding 25 cases and 15 subcohort participants who had missing data on one or more of the serological biomarkers, we included 483 case and 485 subcohort participants in the present analysis.

Data collection and serological biomarkers measurement

At the baseline visit, all participants completed a self-administered questionnaire to provide information on general demographics, medical, smoking, dietary, and occupational histories. Height and weight were measured by trained nurses. The data on aspirin/disprin use for 436 cases and 380 subcohort participants and on family history (first-degree relatives) of colon cancer for 383 cases and 340 cohort participants were collected during the follow-up visit from November 1989 through February 1993. Serum samples were collected from each participant after an overnight fasting at baseline.

Serum CML-AGE and sRAGE levels were measured in duplicate by the Microcoat Biotechnologie Company using the AGE-CML-ELISA kit (Microcoat Biotechnologie Company, Bernried, Germany) and the human sRAGE Quantikine ELISA kit (R&D system Inc, Minneapolis, MN), respectively. The AGE-CML-ELISA kit uses a CML-specific monoclonal antibody (mouse monoclonal 4G9; Alteon Inc, Ramsey, NJ) (13). Total sRAGE includes the C-truncated endogenous secreted form of RAGE (esRAGE), which is a splice variant of RAGE that lacks the transmembrane and cytoplasmic portion of the receptor (14), and proteolytic cleavage forms of RAGE (15). Case and subcohort samples were handled identically and placed randomly on each plate (each batch) in the same proportion, along with 10% blinded phantom quality control samples from a pooled sample. The intra-batch and inter-batch coefficient of variation were 7% and 14% for CML-AGE and 3% and 6% for sRAGE, respectively. Serum concentrations of glucose and insulin were determined in 144 cases and 392 sub-cohort participants in a previous investigation (16). In the present study, these two analytes were measured on an additional 364 cases that occurred after 1997 and 100 subcohort participants using the same method in the same laboratory as the earlier study. A pilot study using previously tested 19 samples showed the mean concentration of glucose was lower than the previous test and the mean concentration of insulin was higher than the previous test (*P* values for Wilcoxon signed rank test was 0.10 for glucose and 0.03 for insulin).

Statistical analysis

The distributions of the selected characteristics between the cases and the subcohort participants were compared using the Wilcoxon rank-sum test for continuous variables and the χ^2 test for categorical variables. The residual method was used to adjust dietary intakes for total energy intake. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. The correlation between CML-AGE and sRAGE and with other exposures was examined using Spearman's rank correlation coefficients in the subcohort.

We used weighted Cox proportional hazard regression models to calculate relative risks (RR) and 95% confidence intervals (CI) for colorectal cancer, as well as to perform significance tests for trends and interactions. The quintile cut-points for serological biomarkers were determined based on the distribution among the subcohort participants and the lowest quintile was the reference category. The quartile cut-points were used in sensitivity analyses when the sample size became small. We used the follow-up time as the underlying time metric. The assumption of proportional hazards was tested by generating the interaction term of each exposure variable and person-years of follow-up in the model. In the weighted analysis, case participants were given a sample weight of 1 because they were sampled with certainty, while subcohort participants were given a sample weight of 49.4 (24,708/500). In addition, we evaluated the association between the CML-AGE and colorectal cancer risk using the value adjusted for sRAGE using the residual method because sRAGE was thought to neutralize circulating CML-AGE. The adjusted CMLAGE was used in all the analyses. Potential confounding factors included all the variables in Table 1 and randomization group (α-tocopherol, β-carotene, α-tocopherol and β-carotene, or placebo). Confounding effect was evaluated using backward and forward methods with a variable included in the models if they changed the risk estimates > 10%. None of the variables changed the risk estimate for CML-AGE or sRAGE by more than 10%; however, we included age, years of smoking, and BMI in all models. We further included CML-AGE and sRAGE mutually and adjusted serum insulin and glucose in the models to assess whether the associations between CML-AGE or sRAGE and colorectal cancer were independent of other analytes. We found that the adjustment for serum glucose changed the risk estimates for CML-AGE and sRAGE by > 10%. Therefore, all other potential confounding factors were re-evaluated in the models included age, years of smoking, BMI, CML-AGE or sRAGE, and serum glucose. To further examine the interrelations among the four serological markers, we estimated the associations between serum glucose and insulin and colorectal cancer risk with adjustment for serum CML-AGE or sRAGE with adjustment for the batch effect. The values of these four serological biomarkers were log-transformed to normalize their distributions when used as continuous variables in the models. Dose-response trends across increasing quintiles (or quartile) of the biomarkers were tested using a score variable based on the median value of each quintile (or quartile).

We evaluated the joint effects (i.e., departure from multiplicative models of interaction) of sRAGE with CML-AGE, insulin, or glucose (all dichotomous) for predicting risk of colorectal cancer. Because glucose levels assayed at two different times had slightly different distributions, specific medians among the subcohort participants were used to dichotomize glucose levels at 99 mg/dl for the earlier time-point and 77 mg/dl for the later time-point. The similar approach was used to dichotomize serum insulin. Interactions were represented as cross-product terms and the statistical significance of the interactions was tested using the Wald tests. Using the same approach, we evaluated the joint effects of CML-AGE or sRAGE with number of years of smoking, red meat intake (all used the median in the subcohort as the cut-off points), BMI (<25 versus ≥ 25), use of aspirin/disprin (yes or no), randomization groups (placebo, α-tocopherol only, β-carotene only, or both), and anatomic subsite (proximal versus distal tumors).

In the sensitivity analyses for the main effect and the joint effects, we restricted the study population to 144 cases and 392 subcohort participants whose serum and insulin levels were measured previously; we excluded participants with self-reported diabetes (n = 34); with family history of colon cancer (n = 23) or the information on family history of colon cancer was missing (n = 245), or participants with less than 10 or 15 years of follow-up. All tests were two-sided and *P* values less than 0.05 indicated statistical significance. SAS 9.0 (SAS institute, Inc, Cary, NC) and SUDAAN software (Research Triangle Park, NC) were used for data analyses.

Results

The follow-up was up to 21 years with a median of 15 years. Selected characteristics of 483 cases and 485 subcohort participants are described in Table 1. Compared with the subcohort participants, cases were older, had less heavy physical activity at work, had longer duration but lower intensity of smoking, and had higher daily intake of red meat and lower daily intake of calcium. CML-AGE and sRAGE were positively correlated (r = 0.48, $P < 0.001$). Serum sRAGE was negatively correlated with serum glucose (r = −0.11, $P = 0.02$) and serum CML-AGE was not correlated with serum glucose. CML-AGE had a weak negative correlation with BMI and a positive correlation with daily glucose intake. None was significantly correlated with red meat intake and years of smoking (data not shown).

The median level (interquartile range) of CML-AGE was 540 (478–631) ng/ml for the cases and 561 (471–668) ng/ml for the subcohort. The median level (interquartile range) for sRAGE was 521 (388–707) pg/ml for the cases and 572 (417–742) pg/ml for the subcohort. The median levels of CML-AGE and sRAGE did not differ significantly between the cases and the subcohort participants (*P* values = 0.12 and 0.08 respectively).

Table 2 shows that higher levels of sRAGE were associated with lower risk of colorectal cancer (fifth compared with first quintile, RR, 0.65; 95% CI, 0.39–1.07; *P* value for trend = 0.03) after adjustment for age, years of smoking, BMI and CML-AGE (model 2). Adjustment for serum glucose strengthened the inverse association (RR, 0.52; 95% CI, 0.30–0.89; *P* value for trend = 0.009). There was no significant association between CML-AGE and colorectal cancer risk. We also estimated the risk using adjusted CML-AGE value in that CML-AGE was adjusted for sRAGE. We observed that the 2nd quintile of adjusted CML-AGE was associated with significantly increased risk of colorectal cancer and the significant risk decreased through the 4th quintile and attenuated for the highest quintile of adjusted CML-AGE. The adjustment for serum glucose strengthened the positive association between CML-AGE and pancreatic cancer by more than 10%. The adjustment for food consumptions, nutrient intake, aspirin/disprin use or family history of colon cancer did not change the risk estimates for CML-AGE and sRAGE by more than 5%. Table 3 shows that the association of sRAGE and CML-AGE held true for both proximal and distal tumors.

We observed the similar magnitude of the positive associations between serum glucose and insulin and risk of colorectal cancer as previously reported (16) among 144 cases and 392 subcohort participants for whom we had these data, after adjustment for the batch effect. Adjustment for CML-AGE increased the RR for glucose slightly, from 1.97 (95% CI, 0.98–3.96) to 2.17 (95% CI, 1.06–4.45, fourth compared with first quartile) (data not shown).

Table 4 shows the statistically significant joint effects of sRAGE with CML-AGE ($P_{interaction} = 0.01$) and with serum insulin ($P_{interaction} = 0.03$) in association with colorectal cancer risk. The *P* value for the interaction between sRAGE and years of smoking was 0.07. Compared with higher sRAGE and lower CML-AGE, lower sRAGE and higher CML-AGE was associated with 73% increased risk of colorectal cancer. Compared with higher sRAGE and shorter years of smoking, lower sRAGE and longer years of smoking was associated with more than one fold increased risk of colorectal cancer. The interaction of sRAGE by insulin was on a sub-multiplicative scale. Compared with higher sRAGE and lower insulin, the risk associated with lower sRAGE and higher insulin was 1.62 (95% CI, 0.99–2.64), which was less than the product of the individual effect of higher insulin (RR, 1.34; 95% CI, 0.81–2.21) and lower sRAGE (RR, 2.20; 95% CI, 1.40–3.45). There were no significant interactions for CML-AGE or sRAGE by BMI, aspirin/disprin use, red meat intakes, anatomic sites or randomization groups (*P* values for interactions ranged from 0.21 to 0.82) in relation to colorectal cancer risk.

The results for CML-AGE and sRAGE and colorectal cancer were similar in the sensitivity analyses. For example, when fifth compared with first quintile of sRAGE, the RR was 0.44 (95% CI, 0.22–0.86; *P* value for trend = 0.02) among 350 cases and 418 subcohort participants who had been followed up for more than 10 years; the RR was 0.51 (95% CI, 0.29–0.89; *P* value for trend = 0.04) among 470 cases and 464 subcohort participants without diabetes at baseline, and the RR was 0.44 (95% CI, 0.22–0.88; *P* value for trend = 0.003) among 361 cases and 328 subcohort participants who had no family history of colon cancer. Among 144 cases and 392 subcohort participants whose glucose and insulin concentrations were assayed at the earlier time point, we saw a statistically nonsignificant associations between sRAGE and colorectal cancer (RR, 0.71; 95% CI, 0.36–1.40; highest compared with lowest quartile, *P* value for trend = 0.30). The *P* value for the sub-multiplicative interaction between sRAGE and insulin was 0.25.

Discussion

In this prospective study among Finnish male smokers, we found no significant association between serum CML-AGE and colorectal cancer risk. Prediagnostic serum sRAGE was inversely associated with colorectal cancer. Moreover, lower sRAGE in combination with higher CML-AGE or longer years of smoking was associated with higher risk of colorectal cancer compared with higher sRAGE in combination with lower CML-AGE or shorter year of smoking. There was sub-multiplicative interaction between serum sRAGE and insulin.

RAGE recognizes a wide range of environmental stressors and plays a role in innate immune responses and inflammation (17). The engagement of RAGE by CML-AGE results in enhanced generation of reactive oxygen species and activation of a diverse array of signaling cascades that lead to propagation of an inflammatory response by activation of nuclear transcription factors (17). Full-length RAGE has been described as a link between chronic inflammation and cancer development (18–20). Blockade of RAGE-high mobility group box-1 (HMGB1) interaction was shown to decrease the growth and metastases of both implanted and spontaneous tumors in susceptible mice (21). The role of RAGE/multiligand in fostering an inflammatory tumor microenvironment was reviewed recently (22). A handful of *in vitro* and animal studies have suggested that RAGE and its ligands play an important role in colorectal carcinogenesis by changing host immunity and tissue microenvironment (23–26). In clinical studies, co-expression of membranous RAGE with HMGB1 has been associated with malignant potential of colorectal adenomas (27) and metastatic potential of colorectal cancer (28).

We observed that risk of colorectal cancer for men with high serum sRAGE was half that for men with low sRAGE and this association was observed for both proximal and distal tumors. Among nondiabetic subjects, higher sRAGE has been associated with lower risks of several age-related chronic diseases (29;30). Among patients with type 2 diabetes, plasma sRAGE was highly inversely correlated with glycemic control, insulin resistance, C-reactive protein, and S100A12 (31). Along the same lines, our findings suggested that by neutralizing RAGE ligands, sRAGE may suppress the creation of a microenvironment for tumor growth fostered by engagement of RAGE and its ligands. Moreover, the significant increased risk of colorectal cancer was observed for men with lower sRAGE in the presence of longer years of smoking or higher CML-AGE. The sub-multiplicative interaction between sRAGE and insulin also supported the involvement of sRAGE in the colorectal carcinogenesis.

It is important to evaluate the associations between AGEs and cancer because of our daily exposure to dietary AGEs (2). AGEs induce permanent abnormalities in the extracellular matrix component and elicit oxidative stress, vascular inflammation, and thrombosis (32).

AGEs can attenuate cellular insulin sensitivity in 3T3-L1 adipocytes (33). One study showed a strong to moderate AGEs staining by immunohistochemistry in colon adenocarcinoma samples and the surrounding fibroblasts of five patients (34). Yamagishi et al (35) hypothesized that AGEs explain the molecular link between hyperglycemia/diabetes and colorectal cancer. Our data do not strongly support this hypothesis because we did not observe a significant trend of increased risk of colorectal cancer with increasing CML-AGE. The lack of an association between CML-AGE and colorectal cancer incidence may be attributable to the detoxification or neutralization of CML-AGE by sRAGE or other receptors of AGEs, such as AGE receptor 1, that detoxify CML-AGE and counter-regulate their pro-oxidant effects (36). Nevertheless, we did observe an increased risk associated moderately higher levels of CML-AGE and sRAGE-adjusted CML-AGE. We speculate when CML-AGE levels were high, more secreted sRAGE counteracts the effect of CML-AGE. The dynamic interaction between AGEs and the receptors in metabolism and their role in colorectal carcinogenesis need further characterization and elucidation.

The present investigation adds to the literatures relating circulating AGEs and sRAGE to several age-related diseases. Interestingly, these two markers are modulated by medications and lifestyle. Circulating levels of sRAGE increased among patients with type 1 diabetes who received angiotensin-converting enzyme inhibitor (ACEi) (37) and among patients with hypercholesterolemia after the treatment with statin (38). Plasma AGEs levels were reduced among women with polycystic ovary syndrome after the treatment with metformin, which is an insulin sensitizer and potent glycation inhibitor (39). ACEi, statin, and metformin have been shown to have anti-cancer properties in observational studies (40–42). Moreover, AGEs levels are modulated by dietary intake (43). The modifiable nature of AGEs and sRAGE potentially will provide opportunity for cancer prevention if their roles in cancer development are elucidated.

This present prospective study has several strengths. The fasting blood samples had been collected at least five years before the diagnosis of colorectal cancer and the follow-up was as long as 21 years; therefore, the results are less likely to be influenced by reverse causation. The exclusion of small number of participants with type 2 diabetes generated the same study findings. The extensive data obtained from the questionnaire allowed us to evaluate many potential confounders. Men with conditions (such as heart diseases and chronic renal failure) previously shown to influence serum sRAGE were not included in the ATBC Study and the observed associations would less likely to be confounded by these conditions. However, it is possible that unrecognized conditions, such as preexisting proinflammatory condition at blood collection, confound the association.

Our study also carries certain limitations. Firstly, the present study did not examine non-CML-AGEs or AGEs precursors in association with colorectal cancer risk. It has been reported that non-CML-AGE levels are associated with both fasting glucose and HbA1c levels in patients with diabetes (44). Secondly, our findings may not be generalizable to non-smokers and women and need to be confirmed in other study populations. Nevertheless, a positive correlation between CML-AGE and sRAGE that we saw has been reported in two Japanese studies (45;46). Thirdly, the data on family history of colon cancer and aspirin/disprin use were not complete for all the study subjects because the information was collected during the follow-up. Future study with such information collected should evaluate their confounding effects adequately. Nevertheless, the incidence of colorectal cancer was low in 1985–1988 in Finland and the related influence on the study finding may not be substantial. Lastly, this single study was not able to sort out the interrelations among CML-AGE, sRAGE, serum glucose, and dietary intakes in association with colorectal cancer. Although the adjustment of glucose changed the observed association by more than 10%, the interaction of sRAGE and glucose was not statistically significant.

In summary, we found that sRAGE was inversely associated with colorectal cancer risk. Although biologically plausible, higher CML-AGE levels were not associated with increased colorectal cancer risk. The role of the RAGE/ligand axis in cancer etiology deserves further investigation.

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The author's responsibilities were as follows – 1) LJ and RZSS designed research; 2) LJ, SJW, RZSS, DA, PRT, BIG and JV conducted research; 3) SJW, PRT, DA and JV provided essential materials; 4) LJ, BIG and RZSS performed data analysis; 5) LJ, PRT and RZSS wrote paper; 6) LJ and RZSS had primary responsibility for final content. All authors read and approved the final manuscript.

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REFERENCES

1. Singh R, Barden A, Mori T, Beilin L. Advanced glycation end-products: a review. Diabetologia. 2001; 44:129–46. [PubMed: 11270668]
2. Uribarri J, Woodruff S, Goodman S, et al. Advanced glycation end products in foods and a practical guide to their reduction in the diet. J Am Diet Assoc. 2010; 110:911–6. [PubMed: 20497781]
3. Cerami C, Founds H, Nicholl I, et al. Tobacco smoke is a source of toxic reactive glycation products. Proc Natl Acad Sci U S A. 1997; 94:13915–20. [PubMed: 9391127]
4. Uribarri J, Cai W, Peppa M, et al. Circulating glycotoxins and dietary advanced glycation endproducts: two links to inflammatory response, oxidative stress, and aging. J Gerontol A Biol Sci Med Sci. 2007; 62:427–33. [PubMed: 17452738]
5. Ramasamy R, Vannucci SJ, Yan SS, Herold K, Yan SF, Schmidt AM. Advanced glycation end products and RAGE: a common thread in aging, diabetes, neurodegeneration, and inflammation. Glycobiology. 2005; 15:16R–28R.
6. Hudson BI, Harja E, Moser B, Schmidt AM. Soluble levels of receptor for advanced glycation endproducts (sRAGE) and coronary artery disease: the next C-reactive protein? Arterioscler Thromb Vasc Biol. 2005; 25:879–82. [PubMed: 15863717]
7. Vazzana N, Santilli F, Cuccurullo C, Davi G. Soluble forms of RAGE in internal medicine 1. Intern Emerg Med. 2009; 4:389–401. [PubMed: 19727582]
8. Jing R, Cui M, Wang J, Wang H. Receptor for advanced glycation end products (RAGE) soluble form (sRAGE): anew biomarker for lung cancer. Neoplasma. 2010; 57:55–61. [PubMed: 19895173]
9. Krechler T, Jachymova M, Mestek O, Zak A, Zima T, Kalousova M. Soluble receptor for advanced glycation end-products (sRAGE) and polymorphisms of RAGE and glyoxalase I genes in patients with pancreas cancer. Clin Biochem. 2010; 43:882–6. [PubMed: 20398646]
10. Tesarova P, Kalousova M, Jachymova M, Mestek O, Petruzelka L, Zima T. Receptor for advanced glycation end products (RAGE)--soluble form (sRAGE) and gene polymorphisms in patients with breast cancer. Cancer Invest. 2007; 25:720–5. [PubMed: 18058469]
11. The alpha-tocopherol, beta-carotene lung cancer prevention study: design, methods, participant characteristics, and compliance. The ATBC Cancer Prevention Study Group. Ann Epidemiol. 1994; 4:1–10. [PubMed: 8205268]

12. Korhonen P, Malila N, Pukkala E, Teppo L, Albanes D, Virtamo J. The Finnish Cancer Registry as follow-up source of a large trial cohort--accuracy and delay. Acta Oncol. 2002; 41:381–8. [PubMed: 12234031]
13. Boehm BO, Schilling S, Rosinger S, et al. Elevated serum levels of N(epsilon)-carboxymethyllysine, an advanced glycation end product, are associated with proliferative diabetic retinopathy and macular oedema. Diabetologia. 2004; 47:1376–9. [PubMed: 15258735]
14. Yonekura H, Yamamoto Y, Sakurai S, et al. Novel splice variants of the receptor for advanced glycation end-products expressed in human vascular endothelial cells and pericytes, and their putative roles in diabetes-induced vascular injury. Biochem J. 2003; 370:1097–109. [PubMed: 12495433]
15. Raucci A, Cugusi S, Antonelli A, et al. A soluble form of the receptor for advanced glycation endproducts (RAGE) is produced by proteolytic cleavage of the membrane-bound form by the sheddase a disintegrin and metalloprotease 10 (ADAM10). FASEB J. 2008; 22:3716–27. [PubMed: 18603587]
16. Limburg PJ, Stolzenberg-Solomon RZ, Vierkant RA, et al. Insulin, glucose, insulin resistance, and incident colorectal cancer in male smokers. Clin Gastroenterol Hepatol. 2006; 4:1514–21. [PubMed: 17162243]
17. Bierhaus A, Nawroth PP. Multiple levels of regulation determine the role of the receptor for AGE (RAGE) as common soil in inflammation, immune responses and diabetes mellitus and its complications. Diabetologia. 2009; 52:2251–63. [PubMed: 19636529]
18. Gebhardt C, Riehl A, Durchdewald M, et al. RAGE signaling sustains inflammation and promotes tumor development. J Exp Med. 2008; 205:275–85. [PubMed: 18208974]
19. Riehl A, Nemeth J, Angel P, Hess J. The receptor RAGE: Bridging inflammation and cancer. Cell Commun Signal. 2009; 7:12. [PubMed: 19426472]
20. Sparvero LJ, safu-Adjei D, Kang R, et al. RAGE (Receptor for Advanced Glycation Endproducts), RAGE ligands, and their role in cancer and inflammation. J Transl Med. 2009; 7:17. [PubMed: 19292913]
21. Taguchi A, Blood DC, del TG, et al. Blockade of RAGE-amphoterin signalling suppresses tumour growth and metastases. Nature. 2000; 405:354–60. [PubMed: 10830965]
22. Rojas A, Figueroa H, Morales E. Fueling inflammation at tumor microenvironment: the role of multiligand/RAGE axis. Carcinogenesis. 2010; 31:334–41. [PubMed: 20028726]
23. Fuentes MK, Nigavekar SS, Arumugam T, et al. RAGE activation by S100P in colon cancer stimulates growth, migration, and cell signaling pathways. Dis Colon Rectum. 2007; 50:1230–40. [PubMed: 17587138]
24. Turovskaya O, Foell D, Sinha P, et al. RAGE, carboxylated glycans and S100A8/A9 play essential roles in colitis-associated carcinogenesis. Carcinogenesis. 2008; 29:2035–43. [PubMed: 18689872]
25. Zen K, Chen CX, Chen YT, Wilton R, Liu Y. Receptor for advanced glycation endproducts mediates neutrophil migration across intestinal epithelium. J Immunol. 2007; 178:2483–90. [PubMed: 17277156]
26. Zill H, Gunther R, Erbersdobler HF, Folsch UR, Faist V. RAGE expression and AGE-induced MAP kinase activation in Caco-2 cells. Biochem Biophys Res Commun. 2001; 288:1108–11. [PubMed: 11700025]
27. Sasahira T, Akama Y, Fujii K, Kuniyasu H. Expression of receptor for advanced glycation end products and HMGB1/amphoterin in colorectal adenomas. Virchows Arch. 2005; 446:411–5. [PubMed: 15789216]
28. Kuniyasu H, Chihara Y, Takahashi T. Co-expression of receptor for advanced glycation end products and the ligand amphoterin associates closely with metastasis of colorectal cancer. Oncol Rep. 2003; 10:445–8. [PubMed: 12579287]
29. Falcone C, Emanuele E, D'Angelo A, et al. Plasma levels of soluble receptor for advanced glycation end products and coronary artery disease in nondiabetic men. Arterioscler Thromb Vasc Biol. 2005; 25:1032–7. [PubMed: 15731496]

30. Pullerits R, Bokarewa M, Dahlberg L, Tarkowski A. Decreased levels of soluble receptor for advanced glycation end products in patients with rheumatoid arthritis indicating deficient inflammatory control. Arthritis Res Ther. 2005; 7:R817–R824. [PubMed: 15987483]

31. Basta G, Sironi AM, Lazzerini G, et al. Circulating soluble receptor for advanced glycation end products is inversely associated with glycemic control and S100A12 protein. J Clin Endocrinol Metab. 2006; 91:4628–34. [PubMed: 16926247]

32. Brownlee M. Advanced protein glycosylation in diabetes and aging. Annu Rev Med. 1995; 46:223–34. [PubMed: 7598459]

33. Unoki H, Bujo H, Yamagishi S, Takeuchi M, Imaizumi T, Saito Y. Advanced glycation end products attenuate cellular insulin sensitivity by increasing the generation of intracellular reactive oxygen species in adipocytes. Diabetes Res Clin Pract. 2007; 76:236–44. [PubMed: 17097186]

34. van Heijst JW, Niessen HW, Hoekman K, Schalkwijk CG. Advanced glycation end products in human cancer tissues: detection of Nepsilon-(carboxymethyl)lysine and argpyrimidine. Ann N Y Acad Sci. 2005; 1043:725–33. [PubMed: 16037299]

35. Yamagishi S, Nakamura K, Inoue H, Kikuchi S, Takeuchi M. Possible participation of advanced glycation end products in the pathogenesis of colorectal cancer in diabetic patients. Med Hypotheses. 2005; 64:1208–10. [PubMed: 15823719]

36. Cai W, He JC, Zhu L, Lu C, Vlassara H. Advanced glycation end product (AGE) receptor 1 suppresses cell oxidant stress and activation signaling via EGF receptor. Proc Natl Acad Sci USA. 2006; 103:13801–6. [PubMed: 16954185]

37. Forbes JM, Thorpe SR, Thallas-Bonke V, et al. Modulation of soluble receptor for advanced glycation end products by angiotensin-converting enzyme-1 inhibition in diabetic nephropathy. J Am Soc Nephrol. 2005; 16:2363–72. [PubMed: 15930093]

38. Santilli F, Bucciarelli L, Noto D, et al. Decreased plasma soluble RAGE in patients with hypercholesterolemia: effects of statins. Free Radic Biol Med. 2007; 43:1255–62. [PubMed: 17893038]

39. amanti-Kandarakis E, Alexandraki K, Piperi C, et al. Effect of metformin administration on plasma advanced glycation end product levels in women with polycystic ovary syndrome. Metabolism. 2007; 56:129–34. [PubMed: 17161235]

40. Ben S,I, Le Marchand-Brustel Y, Tanti JF, Bost F. Metformin in cancer therapy: a new perspective for an old antidiabetic drug? Mol Cancer Ther. 2010; 9:1092–9. [PubMed: 20442309]

41. Boudreau DM, Yu O, Johnson J. Statin use and cancer risk: a comprehensive review. Expert Opin Drug Saf. 2010; 9:603–621. [PubMed: 20377474]

42. Lindberg H, Nielsen D, Jensen BV, Eriksen J, Skovsgaard T. Angiotensin converting enzyme inhibitors for cancer treatment? Acta Oncol. 2004; 43:142–52. [PubMed: 15163162]

43. Vlassara H, Cai W, Crandall J, et al. Inflammatory mediators are induced by dietary glycotoxins, a major risk factor for diabetic angiopathy. Proc Natl Acad Sci USA. 2002; 99:15596–601. [PubMed: 12429856]

44. Takeuchi M, Makita Z, Yanagisawa K, Kameda Y, Koike T. Detection of noncarboxymethyllysine and carboxymethyllysine advanced glycation end products (AGE) in serum of diabetic patients. Mol Med. 1999; 5:393–405. [PubMed: 10415164]

45. Nakamura K, Yamagishi S, Adachi H, et al. Serum levels of sRAGE, the soluble form of receptor for advanced glycation end products, are associated with inflammatory markers in patients with type 2 diabetes. Mol Med. 2007; 13:185–9. [PubMed: 17592553]

46. Yamagishi S, Adachi H, Nakamura K, et al. Positive association between serum levels of advanced glycation end products and the soluble form of receptor for advanced glycation end products in nondiabetic subjects. Metabolism. 2006; 55:1227–31. [PubMed: 16919543]

Table 1

Baseline characteristics of colorectal cancer cases and subcohort participants

Characteristics[a]	Cases N=483	Subcohort N=485	*P* value[b]
General			
Age at randomization, year	57.8 (5.1)	56.4 (5.0)	<0.0001
Body mass index, kg/m^2	26.6 (3.9)	26.7 (4.0)	0.55
Physical activity at work (heavy)	38 (7.8)	45 (9.2)	0.02
Years of smoking	36.4 (8.4)	35.2 (8.2)	0.004
No. of cigarettes smoked per day	19.3 (8.6)	20.8 (8.5)	0.003
History of diabetes mellitus (yes)	13 (2.6)	22 (4.4)	0.17
Family history of colon cancer[c] (yes)	14 (3.7)	9 (2.7)	0.40
Daily aspirin/disprin use[d] (yes)	57 (13)	60 (16)	0.27
Daily dietary or nutrient intakes[e]			
Total meat (g)	202 (75.5)	195 (68.7)	0.13
Red meat (g)	72.6 (32.4)	68.3 (28.2)	0.06
Processed meat (g)	75.7 (54.5)	75.1 (52.4)	0.86
Fruit (g)	221 (193)	218 (182)	0.79
Vegetable (g)	740 (239)	726 (223)	0.34
Total energy (kcal)	2716 (773)	2687 (771)	0.57
Protein (g)	95.2 (13.3)	95.2 (12.7)	0.99
Total fat (g)	101 (16)	101 (15)	0.70
Available carbohydrate (g)	267 (39)	266 (39)	0.82
Glucose (g)	9.48 (5.75)	9.39 (5.77)	0.82
Total fiber (g)	18.5 (9.2)	18.9 (8.6)	0.44
Folate (μg)	342 (61)	344 (61)	0.53
Calcium (mg)	1366 (431)	1438 (453)	0.01
Vitamin D ((μg)	5.7 (3.4)	5.3 (2.8)	0.06
Alcohol (g)	18.6 (20.9)	19.0 (22.7)	0.76

AGEs, advanced glycation end-products; sRAGE, soluble receptor for advanced glycation end-products.

[a] Mean (standard deviation) for the continuous variable and N (%) for the categorical variable.

[b] *P* value based on Wilcoxon rank sum tests or Student's t test for continuous variables and χ^2 tests for categorical variables.

[c] Data on family history of colon cancer (first-degree relatives) collected between 1989–1993 and were available to 380 cases and 340 subcohort participants.

[d] Data on aspirin/disprin use collected between 1989–1993 were available to 436 cases and 380 subcohort participants.

[e] Food and nutrient variables were adjusted for total energy intake using the residual method.

Table 2

Relative risk of colorectal cancer according to quintiles of CML-AGE, sRAGE-adjusted CML-AGE and sRAGE

	Quintiles (Q)					
CML-AGE	**Q1**	**Q2**	**Q3**	**Q4**	**Q5**	***P* value for trend[a]**
Case/subcohort	81/97	136/97	101/97	104/97	61/97	
RR[b] (95% CI)	1.0 (ref)	1.60 (1.05–2.42)	1.16 (0.76–1.78)	1.38 (0.89–2.13)	0.69 (0.43–1.08)	0.12
RR[c] (95% CI)	1.0 (ref)	1.74 (1.13–2.68)	1.22 (0.78–1.90)	1.34 (0.85–2.11)	0.71 (0.44–1.14)	0.13
RR[d] (95% CI)	1.0 (ref)	2.18 (1.34–3.56)	1.49 (0.91–2.42)	1.74 (1.05–2.89)	0.97 (0.55–1.70)	0.62
RR[e] (95% CI)	1.0 (ref)	1.84 (1.05–3.22)	1.45 (0.83–2.53)	1.83 (1.03–3.26)	1.20 (0.64–2.26)	0.51
Adjusted CML-AGE[f]						
Case/subcohort	68/97	134/97	111/97	103/97	67/97	
RR[b] (95% CI)	1.0 (ref)	1.93 (1.26–2.95)	1.62 (1.05–2.49)	1.46 (0.95–2.27)	1.01 (0.64–1.61)	0.70
RR[c] (95% CI)	1.0 (ref)	2.03 (1.31–3.16)	1.63 (1.03–2.56)	1.58 (1.00–2.49)	1.01 (0.63–1.63)	0.67
RR[d] (95% CI)	1.0 (ref)	2.32 (1.44–3.72)	1.89 (1.16–3.08)	1.77 (1.10–2.86)	1.19 (0.71–1.99)	0.91
RR[e] (95% CI)	1.0 (ref)	2.19 (1.26–3.82)	2.19 (1.26–3.80)	1.84 (1.05–3.21)	1.66 (0.92–3.00)	0.17
sRAGE						
Case/subcohort	102/97	116/97	97/97	86/97	82/97	
RR[b] (95% CI)	1.0 (ref)	1.00 (0.66–1.50)	0.83 (0.55–1.26)	0.76 (0.50–1.17)	0.68 (0.44–1.04)	0.03
RR[c] (95% CI)	1.0 (ref)	1.06 (0.69–1.63)	0.91 (0.59–1.41)	0.79 (0.51–1.23)	0.68 (0.44–1.06)	0.03
RR[d] (95% CI)	1.0 (ref)	1.02 (0.65–1.60)	0.87 (0.55–1.38)	0.76 (0.47–1.23)	0.65 (0.39–1.07)	0.03
RR[e] (95% CI)	1.0 (ref)	0.86 (0.52–1.40)	0.70 (0.41–1.18)	0.60 (0.35–1.01)	0.52 (0.30–0.89)	0.009

AGEs, advanced glycation end-products; sRAGE, soluble receptor for advanced glycation end-products.

[a] Calculated by assigning the median value of each quintile as a continuous variable and included in the model.

[b] Model 1: Crude RR.

[c] Model 2: RR was adjusted for age.

[d] Model 3: RR was adjusted for age, years of smoking, BMI, and serum sRAGE (for CML-AGE) or serum CML-AGE (for sRAGE).

[e] Model 4: RR was adjusted for serum glucose in addition to model 3.

[f] The serum CML-AGE was adjusted for sRAGE using the residual method.

Table 3

Relative risk of colorectal cancer according to quintiles of CML-AGE, sRAGE adjusted CML-AGE, and sRAGE stratified by anatomic subsites

	Quintiles (Q)					
Proximal tumor (n=147)	**Q1**	**Q2**	**Q3**	**Q4**	**Q5**	***P* value for trend[a]**
CML Case/subcohort	27/97	38/97	27/97	33/97	22/97	
CML RR[b] (95% CI)	1.0 (ref)	2.22 (0.84–5.88)	1.52 (0.58–3.98)	2.09 (0.80–5.46)	1.96 (0.71–5.39)	0.39
Adjusted CML Case/subcohort	24/97	39/97	26/97	34/97	24/97	
Adjusted CML RR (95% CI)	1.0 (ref)	1.95 (0.94–4.02)	1.53 (0.71–3.30)	1.90 (0.92–3.92)	1.66 (0.75–3.69)	0.22
sRAGE Case/Subcohort	34/97	34/97	32/97	25/97	22/97	
sRAGE RR[b] (95% CI)	1.0 (ref)	0.74 (0.34–1.16)	0.70 (0.31–1.61)	0.60 (0.26–1.39)	0.57 (0.24–1.35)	0.22
Distal tumor (n=324)						
CML Case/subcohort	54/97	95/97	71/97	68/97	36/97	
CML RR[b] (95% CI)	1.0 (ref)	1.83 (0.98–3.42)	1.48 (0.80–2.73)	1.72 (0.91–3.28)	1.02 (0.50–2.10)	0.88
Adjusted CML Case/subcohort	44/97	92/97	83/97	64/97	41/97	
Adjusted CML[b] RR (95% CI)	1.0 (ref)	2.32 (1.24–4.36)	2.57 (1.38–4.77)	1.74 (0.92–3.26)	1.64 (0.83–3.22)	0.29
sRAGE Case/Subcohort	66/97	80/97	62/97	58/97	58/97	
sRAGE RR[b] (95% CI)	1.0 (ref)	0.90 (0.52–1.56)	0.67 (0.37–1.21)	0.62 (0.34–1.11)	0.57 (0.31–1.04)	0.04

AGEs, advanced glycation end-products; sRAGE, soluble receptor for advanced glycation end-products.

[a]Calculated by assigning the median value of each quintile as a continuous variable and included in the model.

[b]RR was adjusted for age, BMI, years of smoking, serum sRAGE or CML-AGE, and serum glucose.

Table 4

Joint effects of serum sRAGE with CML-AGE, insulin and smoking in association with colorectal cancer[a]

sRAGE	CML-AGE[b]	Case (N)	Control (N)	RR[c] (95% CI)
≥ median	< median	125	122	1.0 (ref)
≥ median	≥ median	82	120	0.88 (0.55–1.40)
< median	< median	127	121	1.33 (0.85–2.08)
< median	≥ median	149	122	1.73 (1.10–2.71)
sRAGE	**Insulin**			
≥ median	< median	110	130	1.0 (ref)
≥ median	≥ median	97	112	1.34 (0.81–2.21)
< median	< median	158	108	2.20 (1.40–3.45)
< median	≥ median	118	135	1.62 (0.99–2.64)
sRAGE	**Year of smoking**			
≥ median	< 35	79	128	1.0 (ref)
≥ median	≥ 35	128	114	1.59 (0.88–2.87)
< median	< 35	137	134	2.09 (1.31–3.32)
< median	≥ 35	139	109	2.32 (1.26–4.26)

[a] *P* value for the joint effect was 0.01 for sRAGE and CML-AGE, 0.03 for sRAGE and insulin, 0.07 for sRAGE and years of smoking.

[b] CML-AGE was adjusted for sRAGE using the residual method.

[c] RR was adjusted for age, years of smoking, BMI, and serum glucose for the joint effect with CML-AGE; RR was adjusted for serum CML-AGE in addition for the interaction by insulin and years of smoking.