The associations of advanced glycation end products and its soluble receptor with pancreatic cancer risk: a case-control study within the prospective EPIC Cohort

Grote, Verena A ; Nieters, Alexandra ; Kaaks, Rudolf ; Tjønneland, Anne ; Roswall, Nina ; Overvad, Kim ; Nielsen, Michael R Skjelbo ; Clavel-Chapelon, Françoise ; Boutron-Ruault, Marie Christine ; Racine, Antoine ; Teucher, Birgit ; Lukanova, Annekatrin ; Boeing, Heiner ; Drogan, Dagmar ; Trichopoulou, Antonia ; Trichopoulos, Dimitrios ; Lagiou, Pagona ; Palli, Domenico ; Sieri, Sabina ; Tumino, Rosario ; Vineis, Paolo ; Mattiello, Amalia ; Argüelles Suárez, Marcial Vicente ; Duell, Eric J ; Sánchez, María-José ; Dorronsoro, Miren ; Huerta Castaño, José María ; Barricarte, Aurelio ; Jeurnink, Suzanne M ; Peeters, Petra H M ; Sund, Malin ; Ye, Weimin ; Regner, Sara ; Lindkvist, Björn ; Khaw, Kay-Tee ; Wareham, Nick ; Allen, Naomi E ; Crowe, Francesca L ; Fedirko, Veronika ; Jenab, Mazda ; Romaguera, Dora ; Siddiq, Afshan ; Bueno-de-Mesquita, H Bas ; Rohrmann, Sabine

DOI: https://doi.org/10.1158/1055-9965.EPI-11-1139

The associations of advanced glycation end products and its soluble receptor with pancreatic cancer risk: a case-control study within the prospective EPIC cohort

Verena A Grote[1], Alexandra Nieters[2,1], Rudolf Kaaks[1]*, Anne Tjønneland[3], Nina Roswall[3], Kim Overvad[4], Michael R Skjelbo Nielsen[5], Françoise Clavel-Chapelon[6,7], Marie Christine Boutron-Ruault[6,7], Antoine Racine[6,7], Birgit Teucher[1], Annekatrin Lukanova[1], Heiner Boeing[8], Dagmar Drogan[8], Antonia Trichopoulou[9,10], Dimitrios Trichopoulos[10,11,12], Pagona Lagiou[9,11,12], Domenico Palli[13], Sabina Sieri[14], Rosario Tumino[15], Paolo Vineis[16,17], Amalia Mattiello[18], Marcial Vicente Argüelles Suárez[19], Eric J Duell[20,21], María-José Sánchez[21,22], Miren Dorronsoro[21,23], José María Huerta Castaño[21,24], Aurelio Barricarte[21,25], Suzanne M

[1] Division of Cancer Epidemiology, German Cancer Research Center (DKFZ), Heidelberg, Germany
[2] Centre of Chronic Immunodeficiency, CCI Molecular Epidemiology, University Medical Center Freiburg, Freiburg, Germany
[3] Institute of Cancer Epidemiology, Danish Cancer Society, Copenhagen, Denmark
[4] Department of Epidemiology, School of Public Health, Aarhus University, Aarhus, Denmark
[5] Center for Cardiovascular Research, Aarhus University Hospital, Aalborg, Denmark
[6] INSERM, Centre for Research in Epidemiology and Population Health, U1018, Institut Gustave Roussy, Villejuif, France
[7] Paris South University, UMRS 1018, Villejuif, France
[8] Department of Epidemiology, German Institute of Human Nutrition Potsdam-Rehbruecke, Nuthetal, Germany
[9]WHO Collaborating Center for Food and Nutrition Policies, Department of Hygiene, Epidemiology and Medical Statistics, University of Athens Medical School, Athens, Greece
[10] Hellenic Health Foundation, Athens, Greece
[11] Department of Epidemiology, Harvard School of Public Health, Boston, MA, USA
[12] Bureau of Epidemiologic Research, Academy of Athens, Athens, Greece
[13] Molecular and Nutritional Epidemiology Unit, Cancer Research and Prevention Institute - ISPO, Florence, Italy
[14] Nutritional Epidemiology Unit, Fondazione IRCCS Istituto Nazionale Tumori, Milano, Italy
[15] Cancer Registry and Histopathology Unit, "Civile M.P.Arezzo" Hospital, Ragusa, Italy
[16] HuGeF Foundation, Torino, Italy
[17] School of Public Health, Imperial College London, UK
[18] Department of Clinical and Experimental Medicine, Federico II University, Naples, Italy
[19] Public Health Directorate, Asturias, Spain
[20] Unit of Nutrition, Environment and Cancer, Cancer Epidemiology Research Program, Catalan Institute of Oncology (ICO-IDIBELL), L'Hospitalet de Llobregat, Barcelona, Spain
[21] CIBER Epidemiología y Salud Pública (CIBERESP), Spain
[22] Andalusian School of Public Health, Granada, Spain
[23] Public Health Department of Gipuzkoa, Basque Government, Gipuzkoa ,Spain

Jeurnink[26,27], Petra HM Peeters[28,29], Malin Sund[30], Weimin Ye[31,32], Sara Regner[33], Björn Lindkvist[34,], Kay-Tee Khaw[35], Nick Wareham[36], Naomi E Allen[37], Francesca L Crowe[37,] Veronika Fedirko[38], Mazda Jenab[38], Dora Romaguera-Bosch[39], Afshan Siddiq[39], H Bas Bueno-de-Mesquita[26,27], Sabine Rohrmann[1,40]

***Correspondence to:**

Rudolf Kaaks

Division of Cancer Epidemiology c020

German Cancer Research Center (DKFZ)

Im Neuenheimer Feld 581

69120 Heidelberg

Germany

Telefone: ++49 6221 42 2219

Fax: ++49 6221 42 2203

Email: r.kaaks@dkfz.de

---

[24] Department of Epidemiology, Murcia Regional Health Authority, Murcia, Spain
[25] Navarre Public Health Institute, Pamplona, Spain
[26] Department of Gastroenterology and Hepatology, University Medical Centre Utrecht (UMCU), Utrecht, The Netherlands
[27] National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands
[28] Julius Center, University Medical Center Utrecht, Utrecht, The Netherlands
[29] Department of Epidemiology and Biostatistics, School of Public Health, Faculty of Medicine, Imperial College, London, UK
[30] Departments of Surgical and Perioperative Sciences, Surgery and Public Health and Clinical Medicine, Nutrition Research, Umeå University, Umeå, Sweden
[31] Department of Medical Epidemiology and Biostatistics, Karolinska Institute, Stockholm, Sweden
[32] The Medical Biobank at Umeå University, Umeå, Sweden
[33] Department of Surgery, Institution of Clinical Sciences, Malmö, Scania University Hospital, Lund University, Sweden
[34] Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden
[35] Department of Public Health and Primary Care, University of Cambridge, Cambridge, UK
[36] MRC Epidemiology Unit, Cambridge, UK
[37] Cancer Epidemiology Unit, Nuffield Department of Clinical Medicine, University of Oxford, Oxford, UK
[38] International Agency for Research on Cancer (IARC-WHO), Lyon, France
[39] School of Public Health, Imperial College, London, United Kingdom
[40] Division of Cancer Epidemiology and Prevention, Institute of Social and Preventive Medicine, Zurich, Switzerland

Running title: advanced glycation and pancreatic cancer risk

Key words: AGE, esRAGE, CML, EPIC, cohort study, pancreatic cancer

Word count Text: 3425

Word count Abstract: 245

Number of Tables: 3

Number of Figures: 1

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Financial Support

VAG was funded by a grant from the German Research Foundation, Graduiertenkolleg 793: Epidemiology of communicable and chronic noncommunicable diseases and their interrelationships. This work was supported by WCRF UK and WCRF International, grant no 2009/39. The coordination of EPIC is financially supported by the European Commission (DG-SANCO) and the International Agency for Research on Cancer. 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); Deutsche Krebshilfe, Deutsches Krebsforschungszentrum (DKFZ) and Federal Ministry of Education and Research (Germany); Hellenic Health Foundation and Stavros Niarchos Foundation (Greece); Italian Association for Research on Cancer (AIRC) and National Research Council (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); ERC-2009-AdG 232997 and Nordforsk, Nordic Centre of Excellence programme on Food,

Nutrition and Health. (Norway); Health Research Fund (FIS), Regional Governments of Andalucía, Asturias, Basque Country, Murcia (no. 6236) and Navarra, ISCIII RETIC (RD06/0020) (Spain); Swedish Cancer Society, Swedish Scientific Council and Regional Government of Skåne and Västerbotten (Sweden); Cancer Research UK and Medical Research Council (United Kingdom).

Abbreviations:

AGE: Advanced Glycation Endproduct

BMI: Body-Mass-Index

CI: Confidence Interval

CML: Nε-(carboxymethyl)lysine

CRP: C-reactive protein

ELISA; Enzyme-Linked Immune Sorbent Assay

EPIC: European Prospective Investigation into Cancer and nutrition

esRAGE: endogenous secreted receptor for AGE, a splice variant of RAGE

HbA1c: Glycated haemoglobin

IL-6: Interleukin-6

NF-κB: nuclear factor-kappaB

OR: Odds Ratio

sRAGE: soluble receptor for AGE, circulates after shedding of RAGE

WHR: Waist-Hip Ratio

Abstract (words 245)

Background: Advanced glycation endproducts (AGE) and their receptor (RAGE) have been implicated in cancer development through their pro-inflammatory capabilities. However, prospective data on their association with cancer of specific sites, including pancreatic cancer, are limited.

Methods: Pre-diagnostic blood levels of the AGE product Nε-(carboxymethyl)lysine (CML) and the endogenous secreted receptor for AGE (esRAGE) were measured using ELISA in 454 exocrine pancreatic cancer patients and individually matched controls within the European Prospective Investigation into Cancer and Nutrition (EPIC). Pancreatic cancer risk was estimated by calculating odds ratios (OR) with corresponding 95% confidence intervals (CI).

Results: Elevated CML levels tended to be associated with a reduction in pancreatic cancer risk (OR = 0.57 [95% CI 0.32-1.01] comparing highest with lowest quintile), whereas no association was observed for esRAGE (OR = 0.98 [95% CI 0.62-1.54]). Adjustments for BMI and smoking attenuated the inverse associations of CML with pancreatic cancer risk (OR = 0.78 [95% CI 0.41-1.49]). There was an inverse association between esRAGE and risk of pancreatic cancer for cases that were diagnosed within the first two years of follow-up (OR = 0.46 [95% CI 0.22-0.96] for a doubling in concentration), whereas there was no association among those with a longer follow-up (OR = 1.11 [95% CI 0.88-1.39], p interaction = 0.002).

Conclusions and impact: Our results do not provide evidence for an association of higher CML or lower esRAGE levels with risk of pancreatic cancer. The role of AGE/RAGE in pancreatic cancer would benefit from further investigations.

Introduction

Advanced glycation endproducts (AGE) are formed by non-enzymatic reactions of reduced sugars, such as glucose, with amino groups in proteins, lipids, and nucleic acids. Exogenous sources of AGE are those derived from tobacco and those from thermally processed food, so called dietary AGEs, whereas endogenously, AGEs can be formed in a wide range of body tissues and cell types. The endogenous formation of AGE is slow under non-pathological conditions but enhanced during hyperglycaemia. One of the most prominent and also best studied AGE is Nε-(carboxymethyl)lysine (CML), a glycoxidation product (1, 2). AGEs are thought to exert their pro-inflammatory effects by binding to receptors, with RAGE being the best characterized (2, 3). In blood, soluble forms of RAGE have been detected, including splice variants such as the most prominent endogenous secreted RAGE (esRAGE or RAGEv1, (4)) or cleavage forms of membrane-bound full-length RAGE (5). These soluble forms of RAGE are suspected to bind free AGEs and, therefore, might act as "decoy receptors", preventing RAGE ligands from interaction with cell surface RAGE. Consequently, this is thought to inhibit angiogenesis and tumour cell activation (6).

AGE and RAGE are expressed in many tissues and cell types (2, 7), and for many years, both have been implicated in a number of metabolic, neurodegenerative, and inflammatory diseases such as diabetes mellitus and vascular diseases (8). More recently, involvement of the AGE/RAGE axis in cancer has been suspected (6, 7, 9, 10). Human investigations on AGE and RAGE concentrations and their associations with pancreatic cancer risk are, however, limited. One hospital-based case-control study has shown decreased levels of soluble RAGE among their 51 pancreatic cancer patients compared to cancer-free control subjects (11) and a prospective case-cohort study among male Finnish smokers with 255 cases observed a decrease in risk of pancreatic cancer with elevated soluble RAGE, whereas CML levels were not related to pancreatic cancer risk (12). Up to date, no prospective study has been conducted investigating the relationship of the splice variant esRAGE with risk of pancreatic cancer.

To elucidate the suspected positive relationship of CML and the suspected inverse relationship of esRAGE with risk of pancreatic cancer, we conducted a nested case-control study within the European Prospective Investigation into Cancer and Nutrition (EPIC), using data from 454 pancreatic cancer subjects and the equal number of cancer-free control subjects selected from the general western European population.

Materials and Methods

The EPIC cohort

EPIC is a large prospective cohort study with 519,978 participants enrolled between 1992 and 2000 in 23 centres across 10 European countries (Denmark, France, Germany, Greece, Italy, the Netherlands, Norway, Spain, Sweden, and the United Kingdom). At baseline, blood was taken and detailed questionnaires were distributed, covering amongst others habitual diet, anthropometric measures, data on physical activity, socio-economic status, smoking and medical conditions such as history of diabetes. Study design, population, and baseline data collection have been described previously in detail (13, 14). EPIC as well as this individual project were approved by the local ethical review committees and each study participant provided informed consent.

Ascertainment of cases and control selection

In all EPIC centres, data on vital status are collected through mortality registries, in combination with health insurance data (France) or active follow-up (Greece). Population cancer registries (Denmark, Italy, the Netherlands, Spain, Sweden, and the United Kingdom) or a combination of methods including insurance records, cancer and pathology registries, and active follow-up through study subjects (France, Germany, Greece) were used to identify incident cancer cases. For the present project on pancreatic cancer, closure date of the study period were defined as the latest date of complete follow-up for both cancer incidence and vital status in each EPIC centre; varying from December 2002 to December 2005. The last known contact, the date of diagnosis, or the date of death, whichever came first, were defined as the end of follow-up in Germany, Greece, and France.

Exocrine pancreatic cancer incident data were coded as C25 (25.0-25.3, 25.7-25.9) according to ICD-10. Cases were selected among both sexes who developed pancreatic cancer after their recruitment into the study and before the end of the study period. Exclusion criteria were occurrence of other malignant tumours preceding pancreatic cancer diagnosis,

except non-melanoma skin cancer, and non-availability of blood specimens. Follow-up has led to the identification of 578 primary exocrine pancreatic adenocarcinomas, for 466 of these blood specimens were available, and for 454 sRAGE and CML could be measured. Most tumours occurred in the pancreatic head (42%) with few in the body (7%) and tail (5%), while the rest of the tumours were of unknown localization. 333 (73%) of the pancreatic cancer cases were microscopically confirmed and the remaining 34% were diagnosed by physical examination, imaging results, or clinical symptoms. For each case, one control alive and free of cancer at the time of diagnosis of the case was selected using an incidence density sampling procedure. Cases and controls were individually matched, using the following matching criteria: centre, sex, age at blood collection (+/- 3 years), date of blood donation (+/- 3 months), time of blood donation (+/- 2 hours), fasting status (<3 hours, 3-6 hours, >6 hours after last meal) and, in women, use of hormones (oral contraceptive pill, hormone or oestrogen replacement therapy).

Biological samples and laboratory analyses

Blood samples of roughly 420,000 EPIC participants were aliquoted and either stored in liquid nitrogen (-196°C) at a central biorepository, or locally in freezers at -70°C (Sweden) or nitrogen vapour (-150°C, Denmark). Serum samples (and EDTA plasma for the Swedish centre Umea) from one centre were analyzed within the same analytical batch in the specialized immunoassay laboratory of the Division of Cancer Epidemiology (Heidelberg, Germany). ELISA was used to measure esRAGE in serum and plasma samples (B-Bridge International, Inc., Mountain View, CA, USA). Measurements for CML were performed with ELISA from a different company in serum samples only, after enzymatic pre-treatment with Proteinase K (Synvista Therapeutics, Inc., Montvale, NJ, USA). Therefore, plasma samples from Umea were not included in the CML analysis. In total, esRAGE could be measured for 886 subjects and CML for 832 subjects. Intra-batch and inter-batch coefficients of variation were 2.2 and 5.2% for esRAGE and 10.6 and 19.4% for CML. Units for esRAGE are expressed as pg/mL and for CML as ng/mL.

Statistical analyses

Partial Spearman's rank correlation coefficients [ρ] adjusted for age, sex, and EPIC recruitment centre were used to assess the correlations of CML and esRAGE with anthropometric measures, glycated haemoglobin (HbA1c), age, and other variables of interest such as dietary factors or inflammatory markers. HbA1c and inflammatory markers have been measured previously in the same study population and the results on HbA1c have been published recently (15).

Conditional logistic regression models were applied to estimate odds ratios (OR) and 95% confidence intervals (CI) for the associations of CML and esRAGE with pancreatic cancer risk, by either modelling the exposure variables continuously or using quintiles. Cut-points for the categories were based on the distribution of controls and trend tests over quintiles were assessed by modelling the median value within each category as a continuous variable. Continuous measurements of CML and esRAGE were log-transformed to achieve approximate normality. To assess the association of bioavailable CML with risk of pancreatic cancer, we calculated the CML / esRAGE ratio and used this variable in all statistical models in addition to CML and esRAGE.

Conditional logistic regression was also used to investigate the relationship of potential confounders with cancer risk, including body mass index (BMI), waist circumference, waist-hip ratio (WHR), dietary factors (daily average intakes of alcohol, total fat, total protein, carbohydrates, glucose, red meat, processed meat), smoking status (never, former, current, unknown), physical activity (Cambridge Index (16): active, moderately active, moderately inactive, inactive), and diabetes. Subjects were classified as diabetics in the current study if they had baseline HbA1c levels ≥ 6.5% and/or self-reported diabetes at recruitment (n = 93). Variables were considered as confounders if they were associated with the exposure and the outcome and if they changed the logistic β-estimate in a multivariate model by more than 10%. We finally adjusted for BMI as a continuous variable and for smoking status categorically, with the following categories: never smoker; former smoker who stopped less

than 10 years ago, former smoker who stopped 10 or more years ago; current smoker with 1-9, 10-19, or ≥ 20 cigarettes per day; and smoking status unknown. In addition, logistic regression models were mutually adjusted for esRAGE and CML. HbA1c was added in further models to explore the potential additional confounding effect of progressively deteriorating glucose tolerance and of diabetes, and to investigate the effect of hyperglycaemia as an enhancer of AGE-formation.

In addition, analyses were stratified by factors that could modify the relationship between CML and esRAGE and pancreatic cancer, such as diabetes. Heterogeneity of effect was assessed by adding cross-product terms into the logistic regression models over continuous levels of CML and esRAGE and testing the significance with the Wald test, crude and adjusted for BMI and smoking.

All statistical analyses were conducted using the Statistical Analysis System (SAS) software package, Version 9.2 (SAS Institute Inc., Cary, North Carolina, USA). All statistical tests were two-tailed and threshold of significance was 0.05.

Results

Pancreatic cancer cases were on average 63 years old and had a mean follow-up time of 5.3 years (range 0-13; **Table 1**). At baseline, a higher proportion of cases reported being diabetic (14 vs. 8%) or current smokers (31 vs. 22%) compared to controls. No case-control differences by waist-hip ratio were observed among men and women, and no differences by BMI or waist circumference among men. Female pancreatic cancer cases had higher BMI and larger waist circumference than female controls.

CML and esRAGE correlated weakly but significantly with each other among controls (ρ = 0.15 [95% CI 0.04 to 0.24]), and both markers correlated negatively with anthropometric measures with partial correlation coefficients up to -0.27 (**Table 2**). Negative correlations were also observed for esRAGE and CML with inflammatory markers such as C-reactive protein (CRP, Table 2). Controls with diabetes had lower CML (geometric mean = 689 ng/mL [95% CI 625-760]) and esRAGE levels (372 pg/mL [321-432]) than non-diabetic controls (737 [719-755] and 457 [443-437], respectively). CML levels were lowest among current smokers compared with non-smokers but no differences in esRAGE levels were observed by smoking status (data not shown). CML and esRAGE levels correlated significantly with daily average intakes of glucose (Table 2) but not with intakes of total fat, total protein, red meat, processed meat, or alcohol use (data not shown).

Elevated CML levels tended to be associated with a reduced pancreatic cancer risk (crude OR = 0.57 [95% CI 0.32-1.01] comparing highest vs. lowest quintile, p-trend = 0.05; **Table 3**). Adjustments for smoking, BMI, HbA1c, and esRAGE attenuated the association of CML with pancreatic cancer risk (OR = 0.78 [95% CI 0.41-1.49], p-trend = 0.483], with BMI having the strongest effect. By contrast, esRAGE levels were not associated with pancreatic cancer risk (crude OR = 0.98 [95% CI 0.62-1.54], comparing highest with lowest quintile). Additional adjustments for inflammatory markers or dietary factors had a negligible effect on the risk estimates for CML and esRAGE (data not shown). The CML / esRAGE ratio was not associated with risk of pancreatic cancer (Table 3).

The associations of elevated CML and esRAGE levels with pancreatic cancer risk differed statistically significantly by diabetes and smoking status. The association with esRAGE was also modified by follow-up time (time between recruitment [blood collection] and date of tumour diagnosis, **Figure 1**). In these analyses, never and former smokers seemed to have a lower and current smokers a higher risk of pancreatic cancer with elevated CML levels. For higher concentrations of both biomarkers, risk was stronger among diabetic than among non-diabetic participants. However, none of the risk-estimates were significant in either crude or adjusted analyses. For higher esRAGE levels, pancreatic cancer risk was significantly lower by 54% among participants with less than two years of follow-up, which was slightly stronger after multivariate adjustments, whereas there was no association among those with a longer follow-up. No heterogeneity of effects was seen by measures of adiposity, by fasting status, or by median intake of dietary exposures such as red or processed meat (data not shown). The association between CML and pancreatic cancer risk did not differ by levels of esRAGE and vice versa (data not shown).

Discussion

Prospectively, we observed a roughly 40% decrease in pancreatic cancer risk with elevated CML levels (>1017 vs. <560 ng/L), which disappeared after multivariate adjustments for HbA1c levels, BMI, smoking status, and esRAGE concentrations. Elevated esRAGE levels and the CML / esRAGE ratio were not associated with risk of pancreatic cancer.

RAGE and its ligands have been linked to several diseases, including diabetes and its complications, chronic inflammatory diseases (8), and more recently, to cancer (6, 7, 9, 10). Activation of the multiligand/RAGE axis has been shown to perpetuate inflammation at the tumour microenvironment level, stimulate angiogenesis, and support invasion and metastasis (10). In particular, the RAGE ligand S100P and RAGE itself have been found to be over-expressed in pancreatic cancer cells or tissue (7). By contrast, overexpression of the splice variant esRAGE has been shown to inhibit expression of genes involved in tumour formation, cell invasion, and angiogenesis in various cells (17). However, data on CML and esRAGE levels in pancreatic cancer cells or plasma are sparse.

Ours is the third prospective study investigating the association of circulating levels of CML with risk of cancer. Jiao et al. did not detect any associations of CML levels with risk of colorectal cancer (18), but they observed a decrease in risk of pancreatic cancer in the unadjusted analyses, which became non-significant in the multivariable model (12). Our results are in line with the latter, with a borderline inverse association in the crude model. In contrast to our results of esRAGE, we did not observe any effect modification by follow-up time. Based on these results and the observation that plasma AGE levels not necessarily reflect tissue AGE levels (19), plasma levels of CML, and probably also of other AGE, might be elevated in tumour tissue only, but not in plasma either many years prior to tumour diagnosis or shortly before its onset. Future studies are needed to clarify this hypothesis as, to date, no data is available on CML levels in pancreatic tumour tissue and only little is known on plasma circulating levels preceding cancer diagnosis.

The mechanisms by which esRAGE versus RAGE production is regulated are not fully understood (6). Secretion of esRAGE is a consequence of RAGE mRNA processing, possibly indicating enhanced RAGE expression through increased extracellular ligand binding or through intracellular activation of NF-κB secondary to cytokine release. Increasing esRAGE levels have been postulated to bind AGE and, therefore, prevent the activation of post-RAGE signalling (20). Targeted knockdown of RAGE in pancreatic tumour cells resulted in increased apoptosis, diminished autophagy, and decreased tumour cell survival while overexpression had the opposite effects (21). Lower sRAGE and esRAGE levels in tissue, by contrast, have been associated with various tumorigenic states (6). Whether plasma levels reflect tissue levels is not known. But if so, esRAGE (and sRAGE) levels should be decreased in blood of pancreatic cancer patients. Indeed, this has been observed for sRAGE in one case-control study (11). In a prospective setting, this should translate into a decrease in pancreatic cancer risk with higher esRAGE levels, which we did not observe in our study. However, restricting the analyses to subjects developing pancreatic cancer within the first two years of follow-up (time between blood collection and tumour diagnosis), a risk decreasing effect was observed with higher esRAGE levels (OR = 0.46). Whether an undiagnosed tumour has led to lower esRAGE levels or whether lower esRAGE levels have been involved in pancreatic tumour development remains unanswered and needs to be addressed in further prospective studies with a large number of pancreatic cancer cases.

The overall association of pancreatic cancer risk with increasing CML and esRAGE levels seemed to be modified by the diabetes status of our subjects, i.e., a rather strong increase in risk among diabetics and a null-finding among non-diabetic participants. However, confidence intervals were wide and the test for heterogeneity was no longer significant after multivariate adjustments for BMI and smoking status. The first implicates low statistical power and the latter questions the observed effect modification by diabetes status. A recent population based case-control study found higher blood levels of AGE and an increased RAGE expression but a decreased esRAGE expression and lower blood levels of

esRAGE in diabetic than non-diabetic participants (22). Our results are in line with the latter, i.e. lower esRAGE levels in diabetics. We have no explanation for our findings of lower CML levels in diabetic participants, as this is in contrast to the above study and also in contrast to the biological explanation of CML formation. After additional adjustment for HbA1c, ORs for pancreatic cancer risk with increasing CML and esRAGE levels were further off levels of significance in our study. Based on these observations, we suspect that elevated glucose levels and diabetes, but not altered CML and esRAGE levels, are the conditions associated with pancreatic cancer risk.

Our observed negative correlations of CML with anthropometric indices are in line with a recent publication (23). Semba et al. proposed biological mechanisms for the correlations of CML with anthropometry, such that CML might be preferentially stored in fat tissue or metabolized in adipocytes. As overweight correlates with inflammatory markers, we suspected that the observed correlations of CML and esRAGE with inflammatory markers might be confounded by overweight, and, indeed, adjustments for BMI weakened these correlations. Therefore, the known link between overweight and inflammation might be the explanation for the observed correlations of CML with inflammatory markers.

Our study has several limitations. Drawing blood once does not necessarily reflect long-term levels of AGE products and its soluble receptor and repeated freeze-thaw cycles such as in our study might affect CML and esRAGE concentrations in serum/plasma. Moreover, analysing the agents in duplicate was not possible due to limited sample volume. Analyzing the matched case-control set within one assay, however, reduces to some extent laboratory measurement error. Matching on several variables bears the risk of overmatching and thus, may harm statistical efficiency, validity, and/or cost efficiency (24). However, the chosen variables are either standardization variables (age, sex), reflect the variation in incidence of pancreatic cancer (study centre) or the differences in exposure levels at one point in time (fasting), and, therefore, are less likely to have introduced overmatching. If so, then rather an underestimation of the true risk may have occurred by using fasting status. A

further limitation of our study is related to available questionnaire data and its impact on statistical analyses and interpretability of results. An inverse relationship has been observed between renal capacity and circulating CML and esRAGE concentrations in blood (25). We had no information on renal function or related diseases and, thus, could not control for this possible confounder. Our study is the largest with respect to the number of pancreatic cancer subjects in a prospective setting so far, analyzing the risk relationship of CML and esRAGE with primary exocrine adenocarcinoma of the pancreas. And it is the first to investigate esRAGE, as Jiao et al. analyzed sRAGE in their study of male Finnish smokers. sRAGE resembles membrane-bound full-length RAGE, esRAGE, and other splice variant forms of RAGE. We decided to measure esRAGE instead of sRAGE, as the first has been shown to be more stable over time than the latter and, in addition, only esRAGE was found to be capable of capturing AGE-ligands but not other splice variants such as N-truncated RAGE (20). Investigating esRAGE in human cells and organs is a new evolving area of research and only little is known on its impact on diseases and disorders (6, 20, 26).

Future epidemiological studies should analyze several AGE and RAGE derivatives in blood drawn several times, limit the number of freeze-thaw cycles, and collect additional health and disease related information such as kidney function or diseases, diabetes, cardiovascular diseases, or use of therapeutics which may influence circulating blood levels of AGEs or soluble RAGE derivates.

Conclusion

In our study conducted among the general western European population, we did not find a clear association of elevated CML or the endogenous secretory receptor esRAGE with risk of pancreatic cancer. Because only two prospective studies investigated this relationship but with different soluble RAGE derivates, and one of them exclusively among male smokers, further studies are needed to evaluate the potential role of the AGE/RAGE axis with pancreatic cancer risk.

Acknowledgments

We thank Dr. Susen Becker and Miss Britta Lederer for their excellent work in managing and performing the immunoassays.

Grant Support

VAG was funded by a grant from the German Research Foundation, Graduiertenkolleg 793: Epidemiology of communicable and chronic noncommunicable diseases and their interrelationships. This work was supported by WCRF UK and WCRF International, grant no 2009/39. The coordination of EPIC is financially supported by the European Commission (DG-SANCO) and the International Agency for Research on Cancer. 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); Deutsche Krebshilfe, Deutsches Krebsforschungszentrum (DKFZ) and Federal Ministry of Education and Research (Germany); Hellenic Health Foundation and Stavros Niarchos Foundation (Greece); Italian Association for Research on Cancer (AIRC) and National Research Council (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); ERC-2009-AdG 232997 and Nordforsk, Nordic Centre of Excellence programme on Food, Nutrition and Health. (Norway); Health Research Fund (FIS), Regional Governments of Andalucía, Asturias, Basque Country, Murcia (no. 6236) and Navarra, ISCIII RETIC (RD06/0020) (Spain); Swedish Cancer Society, Swedish Scientific Council and Regional Government of Skåne and Västerbotten (Sweden); Cancer Research UK and Medical Research Council (United Kingdom).

References

1. Bierhaus A, Hofmann MA, Ziegler R, Nawroth PP. AGEs and their interaction with AGE-receptors in vascular disease and diabetes mellitus. I. The AGE concept. Cardiovasc Res. 1998;37:586-600.

2. Singh R, Barden A, Mori T, Beilin L. Advanced glycation end-products: a review. Diabetologia. 2001;44:129-46.

3. Mendez JD, Xie J, Aguilar-Hernandez M, Mendez-Valenzuela V. Trends in advanced glycation end products research in diabetes mellitus and its complications. Mol Cell Biochem. 2010;341:33-41.

4. Hudson BI, Carter AM, Harja E, Kalea AZ, Arriero M, Yang H, et al. Identification, classification, and expression of RAGE gene splice variants. The FASEB journal : official publication of the Federation of American Societies for Experimental Biology. 2008;22:1572-80.

5. Santilli F, Vazzana N, Bucciarelli LG, Davi G. Soluble forms of RAGE in human diseases: clinical and therapeutical implications. Current medicinal chemistry. 2009;16:940-52.

6. Kalea AZ, Schmidt AM, Hudson BI. Alternative splicing of RAGE: roles in biology and disease. Frontiers in bioscience : a journal and virtual library. 2011;17:2756-70.

7. Logsdon CD, Fuentes MK, Huang EH, Arumugam T. RAGE and RAGE ligands in cancer. Curr Mol Med. 2007;7:777-89.

8. 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.

9. Abe R, Yamagishi S. AGE-RAGE system and carcinogenesis. Curr Pharm Des. 2008;14:940-5.

10. Rojas A, Figueroa H, Morales E. Fueling inflammation at tumor microenvironment: the role of multiligand/RAGE axis. Carcinogenesis. 2010;31:334-41.

11. 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.

12. Jiao L, Weinstein SJ, Albanes D, Taylor PR, Graubard BI, Virtamo J, et al. Evidence that serum levels of the soluble receptor for advanced glycation end products are inversely associated with pancreatic cancer risk: a prospective study. Cancer Res. 2011;71:3582-9.

13. Haftenberger M, Lahmann PH, Panico S, Gonzalez CA, Seidell JC, Boeing H, et al. Overweight, obesity and fat distribution in 50- to 64-year-old participants in the European Prospective Investigation into Cancer and Nutrition (EPIC). Public Health Nutr. 2002;5:1147-62.

14. Riboli E, Hunt KJ, Slimani N, Ferrari P, Norat T, Fahey M, et al. European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. Public Health Nutr. 2002;5:1113-24.

15. Grote VA, Rohrmann S, Nieters A, Dossus L, Tjonneland A, Halkjaer J, et al. Diabetes mellitus, glycated haemoglobin and C-peptide levels in relation to pancreatic cancer risk: a study within the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort. Diabetologia. 2011.

16. Wareham NJ, Jakes RW, Rennie KL, Schuit J, Mitchell J, Hennings S, et al. Validity and repeatability of a simple index derived from the short physical activity questionnaire used in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. Public Health Nutr. 2003;6:407-13.

17. Kalea AZ, See F, Harja E, Arriero M, Schmidt AM, Hudson BI. Alternatively spliced RAGEv1 inhibits tumorigenesis through suppression of JNK signaling. Cancer Res. 2010;70:5628-38.

18. Jiao L, Taylor PR, Weinstein SJ, Graubard BI, Virtamo J, Albanes D, et al. Advanced Glycation End Products, Soluble Receptor for Advanced Glycation End Products, and Risk of Colorectal Cancer. Cancer Epidemiol Biomarkers Prev. 2011.

19. Meerwaldt R, Links T, Zeebregts C, Tio R, Hillebrands JL, Smit A. The clinical relevance of assessing advanced glycation endproducts accumulation in diabetes. Cardiovasc Diabetol. 2008;7:29.

20. Yonekura H, Yamamoto Y, Sakurai S, Petrova RG, Abedin MJ, Li H, 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.

21. Kang R, Tang D, Schapiro NE, Livesey KM, Farkas A, Loughran P, et al. The receptor for advanced glycation end products (RAGE) sustains autophagy and limits apoptosis, promoting pancreatic tumor cell survival. Cell Death Differ. 2010;17:666-76.

22. Tam XH, Shiu SW, Leng L, Bucala R, Betteridge DJ, Tan KC. Enhanced expression of receptor for advanced glycation end-products is associated with low circulating soluble isoforms of the receptor in Type 2 diabetes. Clin Sci (Lond). 2011;120:81-9.

23. Semba RD, Arab L, Sun K, Nicklett EJ, Ferrucci L. Fat mass is inversely associated with serum carboxymethyl-lysine, an advanced glycation end product, in adults. J Nutr. 2011;141:1726-30.

24. Rothman KJ, Greenland S, Lash TL. Design Strategies to Improve Study Accuracy. In: Rothman KJ, Greenland S, Lash TL, editors. Modern Epidemiology. Philadelphia, PA, USA: Lippincott Williams & Wilkins; 2008.

25. Semba RD, Ferrucci L, Fink JC, Sun K, Beck J, Dalal M, et al. Advanced glycation end products and their circulating receptors and level of kidney function in older community-dwelling women. American journal of kidney diseases : the official journal of the National Kidney Foundation. 2009;53:51-8.

26. Cheng C, Tsuneyama K, Kominami R, Shinohara H, Sakurai S, Yonekura H, et al. Expression profiling of endogenous secretory receptor for advanced glycation end products in human organs. Mod Pathol. 2005;18:1385-96.

Table 1. Baseline characteristics of pancreatic cancer cases and matched controls

Variable	Cases (n=454)	Controls (n=452)
% Women (n)	52 (233)	52 (234)
Age at recruitment [y], mean (range)	58 (30 – 76)	58 (30 – 76)
Age at diagnosis [y], mean (range)	63 (37 – 82)	-
Follow-up [y], mean (range)	5.3 (0 – 13)	-
BMI [kg/m^2], mean ± SD		
Male	26.8 ± 3.6	26.7 ± 3.7
Female	26.5 ± 5.0	25.2 ± 4.3
Waist-hip ratio, mean ± SD		
Male	0.95 ± 0.06	0.95 ± 0.06
Female	0.82 ± 0.07	0.81 ± 0.06
Waist circumference [cm], mean ± SD		
Male	96.3± 9.9	96.7 ± 10.2
Female	84.3 ± 12.5	81.2 ± 10.7
Smoking status, n (%)		
Never	162 (36)	197 (44)
Former	144 (32)	150 (33)
Current	143 (31)	100 (22)
Unknown	5 (1)	5 (1)
Alcohol intake at recruitment [g/d]		
Male, geometric mean (95% CI)	12 (10-15)	11 (9-14)
Female, geometric mean (95% CI)	5 (4-6)	4 (4-5)
Fasting status, n (%)		
Fasting (≥ 6 hours)	118 (26)	113 (25)
In between (3 - 6 hours)	78 (17)	76 (17)
Non fasting (< 3 hours)	176 (39)	182 (40)
Unknown	82 (18)	81 (18)
Self-reported diabetes at recruitment, n (%)	33 (7)	19 (4)
Subjects HbA1c ≥ 6.5%, n (%)	54 (12)	29 (6)
Self-reported diabetes or HbA1c ≥ 6.5%, n (%)	59 (14)	34 (8)
Unknown	18 (4)	17 (4)
CML [ng/mL], geometric mean (95% CI)	728 (705-751)	750 (725-775)
esRAGE [pg/mL], geometric mean (95% CI)	452 (432-473)	454 (435-476)
CML / esRAGE ratio, geometric mean (95% CI)	1.61 (1.53-1.70)	1.64 (1.55-1.73)

Table 2: Partial Spearman's rank correlation coefficients (95% CI) of CML and esRAGE with covariates, in control participants [a]

Covariate	CML	esRAGE
esRAGE	**0.15** (0.04 to 0.24)	-
BMI	**-0.15** (-0.25 to -0.05)	**-0.27** (-0.36 to -0.17)
Waist	**-0.20** (-0.30 to -0.10)	**-0.22** (-0.32 to -0.12)
WHR	-**0.23** (-0.32 to -0.13)	**-0.16** (-0.26 to -0.06)
Smoking status [b]	**-0.12** (-0.21 to -0.02)	-0.09 (-0.19 to 0.01)
Numbers of cigarettes smoked [c]	-0.07 (-0.30 to 0.17)	0.20 (-0.04 to 0.42)
Time since quitting smoking [d]	0.12 (-0.06 to 0.29)	**0.19** (0.01 to 0.36)
Fasting status [e]	-0.10 (-0.21 to 0.01)	-0.04 (-0.15 to 0.07)
Diabetes status [f]	**-0.12** (-0.22 to -0.02)	**-0.12** (-0.22 to -0.02)
HbA1c	-0.07 (-0.18 to 0.03)	-0.09 (-0.19 to 0.02)
Adiponectin	**0.21** (0.11 to 0.31)	**0.16** (0.06 to 0.26)
CRP	**-0.26** (-0.36 to -0.16)	**-0.22** (-0.32 to -0.11)
IL-6	**-0.13** (-0.24 to -0.03)	**-0.16** (-0.26 to -0.05)
Dietary glucose	**0.18** (0.08 to 0.28)	**0.12** (0.02 to 0.22)
Processed meat	-0.01 (-0.11 to 0.09)	-0.07 (-0.17 to 0.03)

[a] Partial Spearman's rank correlation coefficients [ρ] adjusted for age, sex and EPIC recruitment centre. CML and esRAGE levels were log transformed to achieve normality.

[b] Never, former, current smoker

[c] Among current smokers

[d] Among former smokers

[e] Non-fasting (<3 hours), in between (3-6 hours), fasting (>6 hours after last meal)

[f] Non-diabetics, diabetics (self-reported diabetes at recruitment baseline and/or HbA1c levels ≥ 6.5%)

Table 3. Relative risk [OR (95% CI)] of pancreatic cancer by quintiles of CML and esRAGE

		Quintiles [a]					*P* trend [b]	OR for a doubling in concentration
		1	2	3	4	5		
CML	Quartile cut-offs [ng/L]	328 - 559	560 - 680	681 - 817	818 - 1017	1018 - 1819		
	No. cases / controls	86 / 82	99 / 83	88 / 82	74 / 83	65 / 82		
	Model 1[c]	1.0	1.09 (0.70-1.69)	0.92 (0.57-1.50)	0.70 (0.42-1.18)	0.57 (0.32-1.01)	0.05	0.70 (0.48-1.02)
	Model 2[d]	1.0	1.14 (0.72-1.80)	1.13 (0.68-1.87)	0.87 (0.50-1.50)	0.77 (0.42-1.41)	0.4	0.85 (0.57-1.28)
	Model 3[e]	1.0	1.19 (0.75-1.88)	1.22 (0.73-2.06)	0.88 (0.50-1.55)	0.79 (0.42-1.46)	0.5	0.88 (0.59-1.33)
	Model 4[r]	1.0	1.10 (0.68-1.78)	1.20 (0.71-2.05)	0.82 (0.46-1.46)	0.78 (0.41-1.49)	0.5	0.86 (0.56-1.32)
esRAGE	Quartile cut-offs [pg/ml]	109 - 308	309 - 401	402 - 521	522 - 671	672 - 1793		
	No. cases / controls	89 / 86	88 / 86	85 / 87	81 / 86	88 / 86		
	Model 1[c]	1.0	0.98 (0.63-1.51)	0.93 (0.60-1.46)	0.90 (0.57-1.41)	0.98 (0.62-1.54)	0.9	0.98 (0.80-1.21)
	Model 2[d]	1.0	1.02 (0.65-1.60)	0.94 (0.59-1.50)	0.96 (0.60-1.55)	1.09 (0.67-1.78)	0.7	1.04 (0.83-1.30)
	Model 3[e]	1.0	1.08 (0.68-1.72)	1.02 (0.63-1.66)	1.15 (0.70-1.88)	1.18 (0.71-1.94)	0.5	1.12 (0.88-1.41)
	Model 4[r]	1.0	0.97 (0.59-1.59)	1.11 (0.66-1.85)	1.25 (0.74-2.11)	1.14 (0.67-1.94)	0.4	1.13 (0.88-1.45)
Ratio	Quartile cut-offs [pg/ml]	0.34 - 1.03	1.04 - 1.43	1.43 - 1.92	1.95 - 2.65	2.66 - 7.55		
	No. cases / controls	82 / 79	77 / 79	91 / 80	75 / 79	71 / 79		
	Model 1[c]	1.0	0.95 (0.60-1.50)	1.12 (0.70-1.79)	0.89 (0.55-1.43)	0.83 (0.50-1.36)	0.4	0.94 (0.77-1.16)
	Model 2[d]	1.0	0.87 (0.53-1.41)	1.03 (0.63-1.68)	0.86 (0.52-1.43)	0.80 (0.48-1.36)	0.4	0.94 (0.75-1.17)
	Model 3[e]	1.0	0.90 (0.55-1.48)	1.01 (0.60-1.69)	0.81 (0.48-1.36)	0.71 (0.41-1.22)	0.2	0.88 (0.70-1.10)

[a] Quintile cut points were based on the distribution of controls.

[b] *P* trend test was based on median values of each quartile.

[c] Model 1: Crude OR based on logistic regression conditioned on matching factors (sex, age, date and time of blood donation, fasting status, and use of hormones).

[d] Model 2: OR adjusted for smoking and BMI.

[e] Model 3: As in model 2 and additionally adjusted for levels of HbA1c.

[f] Model 4: As in model 3 and additionally adjusted for levels of esRAGE (for CML) or CML (for esRAGE).

CI = confidence interval, No. = number, CML and esRAGE concentrations on continuous scale were log transformed to achieve normality, smaller number of subjects due to missing laboratory values.

Legends of Tables and Figures

Table 1. Baseline characteristics of pancreatic cancer cases and matched controls

Table 2: Partial Spearman's rank correlation coefficients (95% CI) of CML and esRAGE with covariates, among pancreatic cancer cases and control subjects

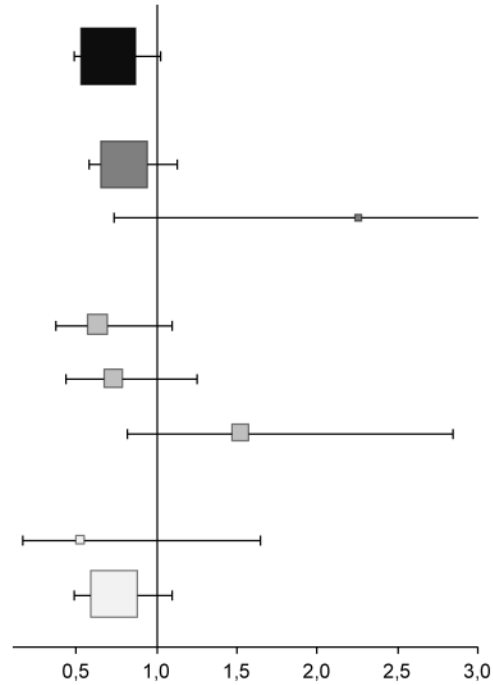
Table 3. Relative risk [OR (95% CI)] of pancreatic cancer by quintiles of CML and esRAGE

Figure 1: Relative risks [OR (95% CI)] of pancreatic cancer for a doubling in CML and esRAGE concentrations, all and stratified by diabetes and smoking status, and by length of follow-up (≤ vs. > 2yrs)

Figure 1: Relative risks [OR (95% CI)] of pancreatic cancer for a doubling in CML and esRAGE concentrations, all and stratified by diabetes and smoking status, and by length of follow-up (≤ vs. > 2yrs) [a]

a) CML, crude

Subgroup	Ca / Co	OR (95% CI)	P int [b]
All	431 / 431	0.70 (0.48-1.02)	
Non-diabetics	340 / 365	0.80 (0.57-1.12)	**0.005**
Diabetics [c]	59 / 33	2.25 (0.73-6.89)	
Never smoker	141 / 174	0.63 (0.37-1.09)	**0.003**
Former smoker	137 / 143	0.73 (0.43-1.25)	
Current smoker	134 / 93	1.52 (0.81-2.84)	
FUP ≤ 2yrs [d]	71 / 71	0.52 (0.16-1.64)	0.6
FUP > 2yrs	341 / 341	0.73 (0.48-1.09)	



b) CML, adjusted

Subgroup	Ca / Co	OR (95% CI)	P int [b]
All	431 / 431	0.88 (0.59-1.33)	
Non-diabetics	340 / 365	0.91 (0.64-1.29)	**0.01**
Diabetics [c]	59 / 33	**5.70 (1.38-23.46)**	
Never smoker	141 / 174	0.71 (0.41-1.23)	0.3
Former smoker	137 / 143	0.82 (0.47-1.42)	
Current smoker	134 / 93	1.69 (0.89-3.22)	
FUP ≤ 2yrs [d]	71 / 71	0.78 (0.22-2.75)	0.7
FUP > 2yrs	341 / 341	0.89 (0.58-1.36)	

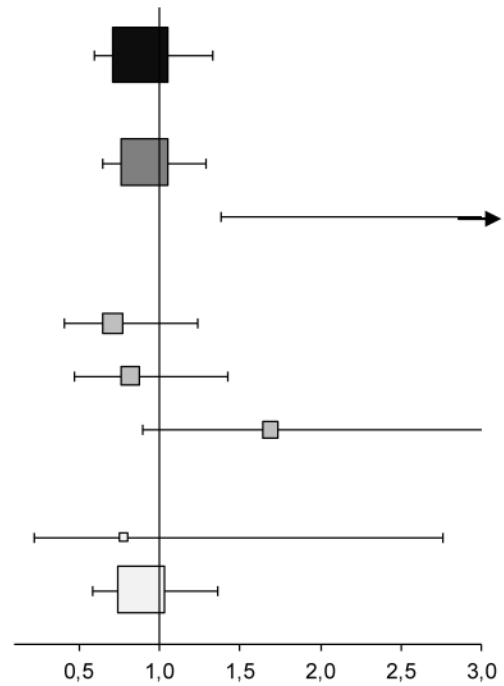
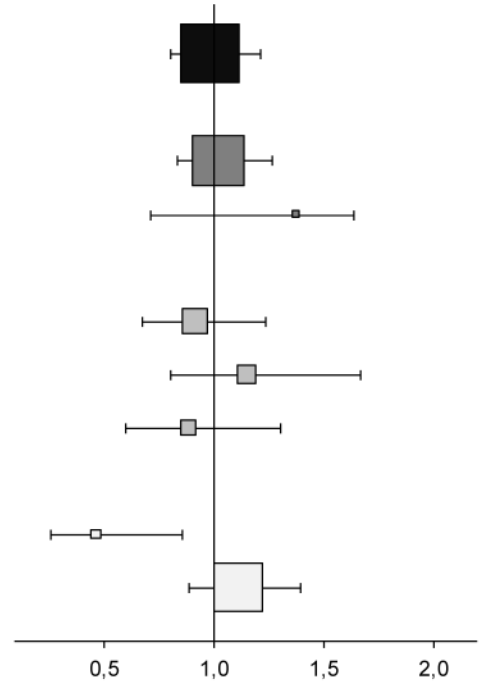


Figure 1 cont.

c) esRAGE, crude

Subgroup	Ca / Co	OR (95% CI)	P int [b]
All	412 / 412	0.98 (0.80-1.21)	
Non-diabetics	373 / 389	1.02 (0.83-1.26)	**0.003**
Diabetics [c]	59 / 31	1.37 (0.71-2.64)	
Never smoker	162 / 193	0.91 (0.67-1.23)	**0.002**
Former smoker	141 / 142	1.15 (0.80-1.66)	
Current smoker	142 / 96	0.88 (0.59-1.30)	
FUP ≤ 2yrs [d]	71 / 71	**0.46 (0.25-0.85)**	**0.009**
FUP > 2yrs	360 / 360	1.11 (0.88-1.39)	



d) esRAGE, adjusted

Subgroup	Ca / Co	OR (95% CI)	P int [b]
All	412 / 412	1.04 (0.83-1.30)	
Non-diabetics	373 / 389	1.05 (0.84-1.31)	**0.01**
Diabetics [c]	59 / 31	2.01 (0.90-4.50)	
Never smoker	162 / 193	1.01 (0.73-1.39)	0.2
Former smoker	141 / 142	1.19 (0.81-1.76)	
Current smoker	142 / 96	0.89 (0.59-1.33)	
FUP ≤ 2yrs [d]	71 / 71	**0.39 (0.19-0.79)**	**0.002**
FUP > 2yrs	360 / 360	1.22 (0.95-1.57)