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

Grote, V A ; Rohrmann, S ; Nieters, A ; Dossus, L ; Tjønneland, A ; Halkjær, J ; Overvad, K ; Fagherazzi, G ; Boutron-Ruault, M C ; Morois, S ; Teucher, B ; Becker, S ; Sluik, D ; Boeing, H ; Trichopoulou, A ; Lagiou, P ; Trichopoulos, D ; Palli, D ; Pala, V ; Tumino, R ; Vineis, P ; Panico, S ; Rodríguez, L ; Duell, E J ; Molina-Montes, E ; Dorronsoro, M ; Huerta, J M ; Ardanaz, E ; Jeurnink, S M ; Beulens, J W J ; Peeters, P H M ; Sund, M ; Ye, W ; Lindkvist, B ; Johansen, D ; Khaw, K T ; Wareham, N ; Allen, N ; Crowe, F ; Jenab, M ; Romieu, I ; Michaud, D S ; Riboli, E ; Romaguera, D ; Bueno-de-Mesquita, H B ; Kaaks, R

Abstract: Our data on HbA(1c) show that individuals who develop exocrine pancreatic cancer tend to have moderate increases in HbA(1c) levels, relatively independently of obesity and insulin resistance-the classic and major risk factors for type 2 diabetes. While there is no strong difference by lag time, more data are needed on this in order to reach a firm conclusion.

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Title pages

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VA Grote[1], S Rohrmann[1,2], A Nieters[1,3], L Dossus[1], A Tjønneland[4], J Halkjær[4], K Overvad[5], G Fagherazzi[6,7], MC Boutron-Ruault[6,7], S Morois[6,7], B Teucher[1], S Becker[1,8], D Sluik[9], H Boeing[9], A Trichopoulou[10,11], P Lagiou[10,12,13], D Trichopoulos[12,13], D Palli[14], V Pala[15], R Tumino[16], P Vineis[17,18], S Panico[19], L Rodríguez[20], EJ Duell[21,22], E Molina-Montes[22,23], M Dorronsoro[22,24], JM Huerta[22,25], E Ardanaz[22,26], SM Jeurnink[27], JWJ Beulens[28,29], PHM Peeters[28.29], M Sund[30], W Ye[31,32], B Lindkvist[33], D Johansen[34], KT Khaw[35], N Wareham[36], N Allen[37], F Crowe[37], M Jenab[38], I Romieu[38], DS Michaud[39,40], E Riboli[39], D Romaguera[39], HB Bueno-de-Mesquita[41], R Kaaks[1]*

Affiliations

1 Division of Cancer Epidemiology, German Cancer Research Center (DKFZ), Heidelberg, Germany
2 Division of Cancer Epidemiology and Prevention, Institute of Social and Preventive Medicine, Zurich, Switzerland
3 Center of Chronic Immunodeficiency, University Medical Center, Freiburg, Germany
4 Institute of Cancer Epidemiology, Danish Cancer Society, Copenhagen, Denmark
5 Department of Epidemiology, School of Public Health, Aarhus University, Aarhus, 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 Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, University Hospital Leipzig, Leipzig, Germany
9 Department of Epidemiology, German Institute of Human Nutrition Potsdam-Rehbruecke, Nuthetal, Germany
10 WHO Collaborating Center for Food and Nutrition Policies, Department of Hygiene, Epidemiology and Medical Statistics, University of Athens Medical School, Athens, Greece
11 Hellenic Health Foundation, Athens, Greece
12 Department of Epidemiology, Harvard School of Public Health, Boston, MA, USA
13 Bureau of Epidemiologic Research, Academy of Athens, Athens, Greece
14 Molecular and Nutritional Epidemiology Unit, Cancer Research and Prevention Institute - ISPO, Florence, Italy
15 Nutritional Epidemiology Unit, Fondazione IRCCS Istituto Nazionale Tumori, Milano, Italy
16 Cancer Registry and Histopathology Unit, "Civile - M. P. Arezzo" Hospital, Ragusa, Italy
17 HuGeF Foundation, Torino, Italy
18 MRC/HPA Centre for Environment and Health, School of Public Health, Imperial College London, UK
19 Department of Clinical and Experimental Medicine, Federico II University, Naples, Italy
20 Public Health and Participation Directorate, Health and Health Care Services Council, Asturias, Spain
21 Unit of Nutrition, Environment and Cancer, Cancer Epidemiology Research Program, Catalan Institute of Oncology (ICO-IDIBELL), L'Hospitalet de Llobregat, Barcelona, Spain
22 CIBER Epidemiología y Salud Pública (CIBERESP), Spain
23 Andalusian School of Public Health, Granada, Spain
24 Public Health Division of Gipuzkoa, Investigation Institute IIS Biodonostia, Health Department Basque Country, Gipuzkoa, Spain

[25] Department of Epidemiology, Murcia Regional Health Authority, Murcia, Spain
[26] Navarre Public Health Institute, Pamplona, Spain
[27] Department of Gastroenterology and Hepatology, University Medical Centre Utrecht (UMCU), Utrecht, 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, 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] Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden
[34] Department of Surgery, Skåne University Hospital, SUS, Malmö, 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, London, UK
[40] Division of Biology and Medicine, Brown University, Providence, RI, USA
[41] Centre for Nutrition and Health (CVG), National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands

*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

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Abstract (247 words)

Aims/hypothesis: There has been long-standing debate whether diabetes is a causal risk factor for pancreatic cancer or a consequence of tumour development. Prospective epidemiologic studies have shown variable relationships of pancreatic cancer risk with blood markers of glucose and insulin metabolism, overall and as a function of lag-times between marker measurements (blood donation) and date of tumour diagnosis.
Methods: Pre-diagnostic levels of HbA1c and C-peptide were measured for 466 pancreatic cancer cases and 466 individually matched controls within the European Prospective Investigation into Cancer and Nutrition. Conditional logistic regression models were used to estimate ORs for pancreatic cancer.
Results: Pancreatic cancer risk gradually increased with increasing pre-diagnostic HbA1c levels up to an OR of 2.42 (95% CI 1.33, 4.39 highest (≥ 6.5%, 48 mmol/mol) vs. lowest (≤ 5.4%, 36 mmol/mol) category), even for subjects with HbA1c levels within the non-diabetic range. C-peptide levels showed no significant relationship with pancreatic cancer risk, irrespective of fasting status. Analyses showed no clear trends towards increasing hyperglycaemia (as marked by HbA1c levels) or reduced pancreatic beta cell responsiveness (as marked by C-peptide levels) with reducing time intervals from blood donation till cancer diagnosis.
Conclusions/interpretation: Our data on HbA1c show that subjects who develop exocrine pancreatic cancer tend to have moderate increases in HbA1c levels, relatively independently of obesity and insulin resistance – the classical and major risk factors for type 2 diabetes. While there is no strong difference by lag time, more data is needed on this to come to a firm conclusion.

Key words: HbA1c, C-peptide, pancreatic cancer, diabetes, EPIC, cohort study

Abbreviations:
EPIC: European Prospective Investigation into Cancer and Nutrition

Introduction

Well-established lifestyle and environmental risk factors for exocrine pancreatic cancer include smoking [1, 2], chronic and acute forms of pancreatitis [3], and excess body weight [4], although the latter is a rather weak risk factor for pancreatic cancer in comparison to several other cancer types [5].

A further, well-documented risk factor for pancreatic cancer is long-standing diabetes, although many studies have also documented the occurrence of diabetes relatively shortly prior to diagnosis of a pancreatic tumour. This adds fuel to the debate as to whether diabetes is a possible cause, or more likely a consequence of tumour development [6, 7]. In relation to the first theory, it has been speculated that elevated plasma glucose levels and/or increased pancreatic insulin secretion, which are both intrinsic to the development of type 2 diabetes, could serve as possible mechanisms explaining the risk association between diabetes and pancreatic tumour development, e.g. by stimulating cellular growth and by inhibiting apoptosis [8, 9]. According to the second theory, diabetes would occur primarily as a result of a developing tumour, which progressively may entrap or invade pancreatic beta cell islets [10], thus causing a gradual destruction of functional beta cell capacity [11], reductions in insulin secretion capacity, and progressive hyperglycaemia.

To further elucidate the possible cause and/or effect relationships between diabetes mellitus and pancreatic cancer, it is important to further elucidate the temporal relationship between pancreatic cancer occurrence and pre-existing disturbances of glucose and insulin metabolism. Previous prospective studies have shown statistically significant associations of pre-diagnostic glucose [12-15] and insulin levels [15, 16] with pancreatic cancer risk, however, results were inconsistent when lag-time was taken into consideration.

Here, we report results from a nested case-control study within the European Prospective Investigation into Cancer and Nutrition (EPIC), measuring pre-diagnostic serum levels of HbA1c and C-peptide in healthy subjects at enrolment, to calculate risk estimates for the association of those markers with pancreatic cancer. To examine the complex, possibly time-dependent relationship of glucose, insulin, diabetes and pancreatic cancer, we additionally stratified our analyses by lag-time between study enrolment (with blood donation) and pancreatic cancer diagnosis.

Research Design and Methods

Study Population

The design and methods of the EPIC study have been described previously [17, 18]. Briefly, 519,978 men and women, mostly aged between 35 and 70 years, were recruited by 23 collaborating centres in 10 European countries (Denmark, France, Germany, Greece, Italy, the Netherlands, Norway, Spain, Sweden, and the United Kingdom) between 1992 and 2000. Ethical review boards of IARC and local institutions gave approval for the study and all participants provided informed consent.

Blood Sample Collection and Storage

In seven EPIC countries (France, Germany, Greece, Italy, the Netherlands, Spain, and the United Kingdom) serum, plasma, erythrocytes, and DNA was aliquoted and stored in liquid nitrogen (-196°C) in a central biorepository. In Denmark blood samples were stored locally in nitrogen vapour (-150°C) and in Sweden in freezers at -70°C. For the present study, Norway was excluded because blood samples had only recently been collected and very few pancreatic cancer cases had been diagnosed after blood donation.

Follow-up for Cancer Incidence and Vital Status

Incident cancer cases were identified by population cancer registries (Denmark, Italy, the Netherlands, Spain, Sweden, and the United Kingdom) or by a combination of methods including health insurance records, cancer and pathology registries, and active follow-up through study subjects (France, Germany, Greece). 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). For the present project on pancreatic cancer, the study period was defined as the latest dates of complete follow-up for both cancer incidence and vital status in each EPIC centre; varying from December 2002 to December 2006. For Germany, Greece, and France, the end of follow-up was considered to be the last known contact, the date of diagnosis, or the date of death, whichever came first.

Selection of Case and Control Subjects

Pancreatic cancer incidence data were coded according to ICD-10 and included all invasive exocrine pancreatic cancers that were coded as C25 (25.0-25.3, 25.7-25.9). Exclusion criteria were occurrence of other malignant tumours preceding the diagnosis of pancreatic cancer, except for non-melanoma skin cancer, and non-availability of blood specimens. By the end of December 2006, 638 incident cases of pancreatic cancer were identified, of which 578 were primary exocrine pancreatic tumours. For 466 of these cases blood specimens were available. Of these, 342 (76%) were microscopically confirmed. For the remaining 24% diagnosis was confirmed by clinical symptoms, imaging results, and/or physical examination. For each case, one control subject, alive and free of cancer at time of diagnosis of the index case, was selected using incidence density sampling. Matching characteristics were study centre sex, age at enrolment (± 6 months), date at entry in the cohort, time between blood sampling and time of last consumption of food~~s~~ and drink~~s~~ (< 3 hours, 3-6 hours, ≥ 6 hours).

Laboratory Assays

Serum or EDTA plasma samples (Swedish centres) from cases and individually matched controls from one centre were analyzed within the same analytical batch. HbA1c was measured in erythrocyte hemolysate with the BioRad Variant Haemoglobin Analyzer at the Karolinska University Laboratory (Karolinska University Hospital, Stockholm, Sweden). Units are expressed as percentages of haemoglobin and in mmol/mol. C-peptide was analyzed by double antibody radioimmunoassay using the RIA DSL-7000 kit (Diagnostic Systems Laboratories Inc., Webster, Texas, USA) at the German Cancer Research Centre, Division of Cancer Epidemiology (Heidelberg, Germany). Units of C-peptide are expressed as ng/ml. Mean intra-batch and inter-batch coefficients of variations were 2.5 % and 4.4 % for HbA1c, and 8.3% and 19% for C-Peptide.

Statistical Analyses

Differences between cases and controls were tested by paired t-tests for continuous variables and by χ^2 tests for categorical variables.

Conditional logistic regression was used to calculate odds ratios (OR) and the corresponding 95% confidence intervals (CI) for the associations between HbA1c and C-peptide and pancreatic cancer risk; HbA1c and C-peptide were computed on a continuous scale and as categorized variables. Continuous measurements of C-peptide were log-transformed to approximate normality, whereas transformation of HbA1c levels had no effect on normality and odds ratios, therefore non-transformed HbA1c values were used in all statistical procedures. Category cut-points were based on the distribution among controls and p-values for trends across categories were based on the median value~~s~~ within each quartile. To assess the association of pancreatic cancer risk among individuals at the highest risk for progression to diabetes (HbA1c ≥ 6.0 (42 mmol/mol) and < 6.5% (48 mmol/mol)) and among

those having diabetes (HbA1c ≥ 6.5% (48 mmol/mol)) [19], the highest HbA1c category was further divided into these two groups. ORs were also computed to assess the association of diabetes with pancreatic cancer risk. In general, subjects were defined as diabetics if they self-reported the condition at recruitment and/or had HbA1c levels ≥ 6.5% (48 mmol/mol) at baseline (n=95).

Conditional logistic regression was also used to assess the association of possible confounders with pancreatic cancer risk other than those controlled for by matching; these include BMI (continuous), waist circumference (continuous), WHR (continuous), alcohol intake at recruitment (continuous), physical activity (active, moderately active, moderately inactive, inactive), smoking status (never, former, current, missing), and diabetes at recruitment (yes, no, missing). Variables remained in the model if they were associated with pancreatic cancer, correlated with HbA1c or C-peptide, or changed the logistic β estimate by more than 10%. We finally adjusted for BMI as a continuous variable and for smoking status as a categorical variable (never smoker; former smoker who stopped less than 10 years ago, former smokers who stopped 10 or more years ago; current smokers with 1-9, 10-19, or ≥ 20 cigarettes per day; smoking status unknown). To explore further possible confounding effects, we additionally adjusted for C-peptide or HbA1c in further models.

In addition, analyses were stratified by factors that could modify the relationship between HbA1c or C-peptide levels and pancreatic cancer. Tests for statistical heterogeneity between subgroups were calculated by adding cross-product terms in the logistic regression models over quartiles of HbA1c and C-peptide and testing the significance with the Wald test, adjusted for confounders.

All statistical analyses were conducted using the Statistical Analysis System (SAS) software package, Version 9.2 (SAS Institute Inc., Cary, North Carolina, USA).

Results

Baseline characteristics of the study population are shown in **Table 1**. Mean follow-up time for cases was 5.3 years (range 0-13 years). Thirty two percent of cases were current smokers compared to 22% of controls, whereas never smokers were more common among controls than among cases. At baseline recruitment, diabetes, either self-reported or defined by elevated HbA1c levels, was more frequent among those who developed pancreatic cancer.

Among women, incident pancreatic cancer cases had a significantly higher BMI and waist circumference than their matched controls but no significant differences in BMI or waist circumference were seen among men (Table 1). Stratifying by smoking and diabetes status, significant case-control differences in BMI and waist circumference appeared to be restricted to never smoking diabetics. Case-control differences in HbA1c were greatest among former smokers, and particularly among former smokers who were also diabetic at baseline ($mean_{cases}$ = 8.1% (65 mmol/mol), $mean_{controls}$ = 6.6% (49 mmol/mol), p-difference = 0.002). C-peptide concentrations were significantly higher among non-fasting (geometric mean = 6.59 [95% CI 6.10, 7.13]) compared to fasting subjects (3.35 [3.04, 3.70]), and among diabetics (6.83 [5.64, 8.27]) compared to non-diabetics (5.07 [4.79, 5.36]). In addition, former smokers (6.24 [5.74, 6.78]) had higher C-peptide levels than current (5.13 [4.62, 5.69]) and never smokers (4.59 [4.21, 5.02]).

Association of diabetes with cancer risk:

Conditional logistic regression analyses showed a clear increase in pancreatic cancer risk overall among diabetic subjects at recruitment (OR = 1.74 [95% CI 1.12-2.71]). This association was particularly strong among subjects with a follow-up time of two years or less

(OR=3.41 [95% CI 1.26-9.20]), as compared to those with a longer follow-up time (OR=1.45 [95% CI 0.89-2.37]), although heterogeneity was not significant (p = 0.227).

Associations of HbA1c with cancer risk:

Increasing HbA1c percentages were associated with a statistically significant increase in pancreatic cancer risk up to an OR of 2.60 comparing highest vs. lowest category (**Table 2**). Multivariate adjustment for BMI, or waist circumference, and smoking status as well as C-peptide levels only slightly attenuated this risk association.

Analyses by lag-time between the date of blood donation (biomarker measurements) and date of pancreatic cancer diagnosis showed no statistically significant heterogeneity of the association of HbA1c with pancreatic cancer risk, although the association was somewhat stronger for subjects with follow-up times above the median length of follow-up of 5.2 years than for those with shorter follow-up times (Table **2**). Also, within less than two years of follow-up the association between HbA1c concentrations and pancreatic cancer risk was of similar strength (adjusted OR = 1.39 [95% CI 0.48, 4.03]) to that seen for longer follow-up times (adjusted OR = 1.57 [95% CI 0.97, 2.54]; p-heterogeneity = 0.038).

Although trend tests over categories of HbA1c did not show a statistically significant heterogeneity by smoking status, stratification by smoking status did reveal a clearly stronger increase in pancreatic cancer risk with increasing HbA1c concentrations for never smokers, a somewhat weaker increase in risk among ex-smokers, and no increase in risk with increasing HbA1c among current smokers (Table 2).

Associations of C-peptide with cancer risk:

Overall, C-peptide concentrations showed no significant relationship with pancreatic cancer risk (**Table 3**). Multivariate adjustments for BMI or waist circumference, smoking categories, or HbA1c, or exclusion of diabetic subjects did not materially change these odds ratio estimates. However, among subjects who had provided a blood sample under fasting conditions (last food consumption ≥ 6 hours ago), increasing C-peptide levels did show a tendency of increasing risks of pancreatic cancer, although risk was not statistically significant (Table 3).

Odds ratio estimates for increasing C-peptide levels did not vary by length of follow-up or smoking status (data not shown).

Further analyses stratified by sex, diabetes status, median BMI or waist circumference did not show statistically significant heterogeneity between these strata for risk associations with HbA1c or C-peptide (data not shown).

Discussion

In this prospective study, we observed an increase in pancreatic cancer risk with increasing pre-diagnostic HbA1c levels, even for subjects with HbA1c levels within the non-diabetic range. The association was only slightly weakened and remained statistically significant after adjustments for BMI or smoking status, and in stratified analyses was most apparent among never smokers. Contrary to HbA1c, pre-diagnostic levels of C-peptide showed no significant associations with pancreatic cancer risk, irrespective of smoking or fasting status. Lag-time analyses showed no clear difference in the association of pancreatic cancer risk with HbA1c or C-peptide levels by longer or shorter follow-up times.

With respect to HbA1c – a relatively stable and long-term marker for blood glucose levels [19] – our data showed evidence of an association of elevated HbA1c concentrations with increased pancreatic cancer risk independently of diabetes. Indeed, the increase in

pancreatic cancer risk was statistically significant as from the third category of HbA1c (5.8-5.9% (40-41 mmol/mol)), which does not include diabetic subjects (≥ 6.5% (48 mmol/mol)) or even individuals at the highest risk for progression to diabetes (≥ 6.0% (42 mmol/mol) and < 6.5% (48 mmol/mol)) based on the definitions of the American Diabetes Association [19]. With few exceptions [20], previous prospective studies have shown statistically significant associations of pre-diagnostic glucose levels with pancreatic cancer risk [12-15], and in these studies, a clear increase in risk was also visible among subjects with intermediate, non-diabetic levels of glycaemia.

Our findings for C-peptide – no significant association with pancreatic cancer risk overall, under fasting or non-fasting conditions – contrast with those from other studies. In a study of four pooled prospective cohorts in the USA, Michaud et al. did observe an increase in pancreatic cancer risk for the highest quartile level of non-fasting plasma C-peptide, but no association with fasting C-peptide [16]. In the Finnish ATBC cohort, by contrast, pancreatic cancer risk was significantly elevated for the highest quartile of fasting serum insulin [15].

Obesity is an important and frequent determinant of insulin resistance and chronic hyperinsulinemia [21], and a very strong risk factor for type 2 diabetes [22]. Paradoxically, however, epidemiologic studies have globally shown only very modest associations of pancreatic cancer risk with BMI or other anthropometric measures of adiposity [4]. Our data showed clear positive correlations of BMI or waist circumference with C-peptide, but very little correlation of these adiposity indices with HbA1c (data not shown), in line with findings from other studies [23, 24].

Adiposity-related insulin resistance, while leading to increased plasma insulin levels, may generally not be the only, or even principal cause for increases in blood glucose levels. Indeed, hyperglycaemia is generally related to a degree of pancreatic beta cell deficiency and insufficient insulin secretion in response to rising glucose levels, and such deficiency is also a central hallmark of all major forms of diabetes. The molecular-pathologic pathways leading to the deficiency vary between different forms of diabetes, and it is conceivable that relatively specific forms of pancreatic pathology are at the origin of the hyperglycaemia in subjects who subsequently develop pancreatic cancer. Based on observations indicating that inflammatory pathways play a very central role in the development of pancreatic cancer [25, 26] while potentially contributing also to the development of diabetes [27], we speculate that such specific pathology could be a form of chronic and low-grade pancreatic inflammation.

Under the hypothesis that, indeed, a chronic and low-grade form of pancreatic pathology is an original cause of hyperglycaemia in subjects who later develop pancreatic malignancies, we examined whether there are signs of moderate and progressive impairment of the pancreatic beta cell responsiveness, as time intervals till diagnosis of a pancreatic tumour get shorter. Our analyses, however, showed only little heterogeneity of the associations of C-peptide and HbA1c levels with pancreatic cancer risk by length of lag-time till cancer diagnosis. Similar lag-time analyses in other prospective studies have led to inconsistent findings so far. In the pooled US cohort [16] and the ATBC cohort [15], there were indications for a stronger association of C-peptide and fasting insulin and glucose levels, respectively, with longer follow-up, whereas two other prospective cohort studies on blood glucose levels and pancreatic cancer risk showed no heterogeneity of these associations by lag-time [12, 13].

A major strength of our nested case-control study is its prospective design, reducing to a certain extent the possibility of reverse causation, whereas a limitation is the single assessment of biochemical indicators, which may be susceptible to biological variation.

In conclusion, the results of our nested-case control study embedded in a large multi-centre cohort support an association of increasing HbA1c levels with pancreatic cancer risk, independent of BMI or diabetes status. However, in contrast to some previous studies, we did not find an association of elevated C-peptide with pancreatic cancer risk. Overall, combining the results from prospective studies so far, data provide no coherent picture in terms of progressive alterations in glucose and/or insulin metabolism at greater or shorter time intervals prior to pancreatic cancer diagnosis. Further research is needed to understand whether elevated plasma glucose is a possible contributing cause for pancreatic cancer, or whether it is a mere epi-phenomenon pointing towards the existence of a moderate pancreatic disorder that predisposes to pancreatic cancer development as well as to diabetes.

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Table 1: Baseline characteristics of pancreatic cancer cases and matched control subjects

Variable	Cases (n=466)	Controls (n=466)	P value[a]
Men, women (n)	225, 241	225, 241	
Age at recruitment (y), mean (range)	58 (30-76)	58 (30-76)	matched
Age at diagnosis (y), mean (range)	63 (37-82)	-	
Follow-up (y), mean (range)	5.3 (0-13)	-	
Smoking status, n (%)			0.005
Never	165 (35)	201 (43)	
Former	147 (32)	158 (34)	
Current	149 (32)	102 (22)	
Unknown	5 (1)	5 (1)	
Alcohol intake at recruitment [g/d], mean ± SD			
Men	21 ± 26	23 ± 30	0.486
Women	9 ± 13	8 ± 11	0.168
Fasting status, n (%)			matched
Fasting (≥ 6 hours)	118 (25)	113 (24)	
In between (3 - 6 hours)	78 (17)	78 (17)	
Non fasting (< 3 hours)	184 (40)	190 (41)	
Unknown	86 (18)	85 (18)	
Self-reported diabetes at recruitment, n (%)	33 (7)	20 (4)	0.065
Subjects HbA1c ≥ 6.5%, n (%)	53 (12)	30 (7)	0.008
Self-reported diabetes or HbA1c ≥ 6.5%, n (%)	59 (13)	36 (8)	0.012
Unknown, n (%)	19 (4)	18 (4)	
BMI [kg/m^2], mean ± SD	26.6 ± 4.3	25.9 ± 4.1	0.007
Men	26.8 ± 3.6	26.7 ± 3.7	0.690
Women	26.4 ± 5.0	25.2 ± 4.3	0.002
Waist circumference [cm], mean ± SD	90.1 ± 12.8	88.5 ± 13.1	0.030
Men	96.4 ± 9.9	96.7 ± 10.1	0.788
Women	84.2 ± 12.5	81.0 ± 10.7	0.001
Waist-hip ratio, mean ± SD	0.88 ± 0.10	0.88 ± 0.10	0.110
Men	0.95 ± 0.06	0.95 ± 0.06	0.501
Women	0.81 ± 0.07	0.81 ± 0.06	0.119
HbA1c [%], mean ± SD	6.01 ± 1.1	5.80 ± 0.6	< 0.0001
C-peptide [ng/ml], geometric mean (95% CI)[b]	5.46 (5.08-5.86)	5.05 (4.67-5.47)	0.161

n = number, y = year. HbA1c mean values in mmol/mol for cases = 42.2, for controls = 39.9

a. *P* values for continuous variables were based on paired t-tests; p values for categorical variables were based on χ^2 tests.

b. C-peptide levels were reported as geometric means, as the distribution of the variable was not normal.

Table 2. Odds ratios (OR) for pancreatic cancer (95% CI) by categories of HbA1c overall and excluding diabetics; and stratified by median follow-up time, median BMI, and smoking status

	Categories[a]					
	1	**2**	**3**	**4**	**5**	**P trend[b]**
Quartile cut-offs [%]	4.8- 5.4	5.5-5.7	5.8-5.9	6.0-6.4	6.5-11.0	
All subjects[c]						
No. cases / controls	72 / 101	131 / 151	102 / 82	97 / 91	54 / 31	
Crude	1.0	1.24 (0.83-1.85)	1.75 (1.14-2.67)	1.59 (1.03-2.45)	2.60 (1.49-4.56)	< 0.001
Adjusted for smoking, BMI	1.0	1.27 (0.84-1.93)	1.77 (1.14-2.75)	1.46 (0.93-2.30)	2.42 (1.33-4.39)	0.002
Excluding diabetic subjects[c,d]						
No. cases / controls	65 / 89	122 / 134	95 / 71	83 / 71	-	
Crude	1.0	1.30 (0.85-1.98)	1.85 (1.18-2.91)	1.68 (1.05-2.70)		0.010
Adjusted for smoking, BMI	1.0	1.42 (0.91-2.21)	1.94 (1.22-3.10)	1.65 (1.01-2.70)		0.020
Median follow-up time (5.2yrs)[c]						
< 5.2 years, no. cases / controls	41 / 46	54 / 75	52 / 44	83 / 63	-	
Crude	1.0	0.89 (0.50-1.60)	1.44 (0.78-2.64)	1.65 (0.90-3.01)		0.069
Adjusted for smoking, BMI	1.0	0.92 (0.50-1.69)	1.37 (0.73-2.56)	1.56 (0.82-2.94)		0.187
≥ 5.2 years, no. cases / controls	33 / 55	77 / 77	51 / 38	68 / 59	-	
Crude	1.0	1.75 (1.00-3.04)	2.23 (1.22-4.07)	1.91 (1.09-3.35)		0.050
Adjusted for smoking, BMI	1.0	1.73 (0.95-3.15)	2.39 (1.26-4.54)	1.74 (0.95-3.18)		0.061
Median BMI (men 26.7, women 24.6)[e]						
< Median, no. cases / controls	40 / 57	65 / 79	48 / 38	51 / 53	-	
Crude	1.0	1.16 (0.68-1.96)	1.75 (0.96-3.19)	1.37 (0.77-2.45)		0.150
Adjusted for smoking	1.0	1.16 (0.67-2.00)	1.41 (0.76-2.63)	1.22 (0.67-2.23)		0.442
≥ Median, no. cases / controls	34 / 44	66 / 73	55 / 44	100 / 69	-	
Crude	1.0	1.25 (0.71-2.21)	1.73 (0.94-3.18)	2.07 (1.18-3.62)		0.004
Adjusted for smoking	1.0	1.27 (0.72-2.27)	1.81 (0.98-3.36)	1.92 (1.08-3.41)		0.013
Smoking status[e]						
Never smoker, no. cases / controls	25 / 54	55 / 64	37 / 35	47 / 44	-	
Crude	1.0	1.84 (1.01-3.37)	2.36 (1.20-4.64)	2.41 (1.26-4.60)		0.008
Adjusted for BMI	1.0	1.76 (0.96-3.23)	2.20 (1.11-4.34)	2.15 (1.11-4.17)		0.025
Former smoker, no. cases / controls	19 / 30	42 / 54	32 / 25	50 / 45	-	
Crude	1.0	1.20 (0.58-2.49)	1.97 (0.88-4.42)	1.73 (0.83-3.60)		0.079
Adjusted for BMI	1.0	1.19 (0.58-2.46)	1.93 (0.86-4.35)	1.65 (0.78-3.49)		0.109
Current smoker, no. cases / controls	29 / 16	34 / 32	33 / 22	51 / 31	-	
Crude	1.0	0.60 (0.27-1.35)	0.85 (0.37-1.97)	0.92 (0.42-2.04)		0.701
Adjusted for BMI	1.0	0.61 (0.27-1.36)	0.85 (0.37-1.97)	0.89 (0.40-1.97)		0.811

No. = number. Smaller number of subjects due to missing laboratory values of HbA1c. HbA1c category ranges (Q) in mmol/mol: Q1 = 28.9-35.5, Q2 = 36.6-38.8, Q3 = 39.9-41.0, Q4 = 42.1-46.4, Q5 = 47.5-96.7.

a. Category cut points of HbA1c were based on the distribution of controls.

b. *P* trend test was based on median values of each category, adjusted for matching factors.

c. Logistic regression conditioned on matching factors (EPIC recruitment centre, sex, age at recruitment, date at entry in the cohort, time between blood sampling and last consumption of foods and drinks). Adjusting variables in further model: smoking (never smoker; former smoker who stopped < 10 years ago, ≥ 10 years ago; current smokers with 1-9, 10-19, or ≥ 20 cigarettes per day; smoking status unknown) and BMI (continuous).

d. Self-reported diabetes at recruitment and/or HbA1c ≥ 6.5% (48 mmol/mol).

e. Unconditional logistic regression, adjusted for matching factors (EPIC recruitment centre, sex, age at recruitment, date at entry in the cohort, time between blood sampling and last consumption of foods and drinks). Adjusting variables in subgroup analysis by median BMI: smoking (never smoker; former smoker who stopped < 10 years ago, ≥ 10 years ago; current smokers with 1-9, 10-19, or ≥ 20 cigarettes per day; smoking status unknown). Adjusting variables in subgroup analysis by smoking status: BMI (continuous).

Note: Uneven number of controls in Hb1c categories due to accuracy of laboratory HbA1c measurements. The laboratory assay measured HbA1c with 1 digit after the comma and this resulted in nodes of subjects with similar HbA1c values. Therefore, it is not possible to equally distribute HbA1c values of controls across the categories. The current distribution is based on a statistical method, which produces equal groups as accurately as possible.In subgroup analyses, HbA1c categories 4 and 5 were collapsed into one category. P interaction based on Wald test, crude p-interaction for median follow-up time = 0.320, median BMI = 0.627, smoking status = 0.604.

Table 3. Odds ratios (OR) for pancreatic cancer (95% CI) by categories of C-peptide overall, excluding diabetic subjects, and by fasting status

	Categories[a]				
	1	**2**	**3**	**4**	**P trend[b]**
Quartile cut-offs [ng/ml]	0.08-2.95	2.99-5.46	5.48-9.26	9.27-19.82	
All subjects					
No. cases / controls	85 / 104	114 / 105	110 / 106	111 / 105	
Crude[c]	1.0	1.42 (0.93-2.16)	1.38 (0.89-2.13)	1.42 (0.90-2.23)	0.299
Adjusted for smoking, BMI	1.0	1.27 (0.82-1.99)	1.16 (0.73-1.83)	1.15 (0.70-1.91)	0.886
Excluding diabetic subjects[d]					
No. cases / controls	78 / 85	95 / 85	86 / 91	86 / 84	
Crude[c]	1.0	1.25 (0.79-1.99)	1.06 (0.66-1.71)	1.15 (0.70-1.90)	0.851
Adjusted for smoking, BMI	1.0	1.23 (0.76-1.99)	1.00 (0.61-1.65)	1.09 (0.64-1.87)	0.957
By fasting status[e]					
Non-fasting, no. cases / controls	18 / 24	41 / 38	46 / 51	65 / 60	
Crude[c]	1.0	1.44 (0.68-3.08)	1.21 (0.58-2.53)	1.44 (0.71-2.94)	0.503
Adjusted for smoking, BMI	1.0	1.45 (0.67-3.13)	1.16 (0.54-2.47)	1.29 (0.62-2.70)	0.807
In between, no. cases / controls	18 / 19	18 / 24	19 / 15	17 / 18	
Crude[c]	1.0	0.80 (0.32-1.96)	1.33 (0.52-3.39)	0.99 (0.39-2.54)	0.770
Adjusted for smoking, BMI	1.0	0.65 (0.25-1.69)	0.85 (0.30-2.39)	0.60 (0.20-1.73)	0.472
Fasting, no. cases / controls	44 / 51	40 / 34	24 / 21	9 / 5	
Crude[c]	1.0	1.39 (0.75-2.60)	1.38 (0.65-2.90)	2.20 (0.65-7.38)	0.186
Adjusted for smoking, BMI	1.0	1.22 (0.61-2.45)	1.22 (0.53-2.83)	1.90 (0.44-8.11)	0.426

No. = number, C-peptide concentrations on continuous scale were log transformed to achieve normality, smaller number of subjects due to missing laboratory values of C-peptide.

a. Category cut points were based on the distribution of controls.

b. *P* trend test was based on median values of each category, adjusted for matching factors.

c. Logistic regression conditioned on matching factors (EPIC recruitment centre, sex, age at recruitment, date at entry in the cohort, time between blood sampling and last consumption of foods and drinks). Adjusting variables in further models: smoking (former smokers adjusted for quitting smoking (< 10 or ≥ 10 years ago), current smokers adjusted for number of cigarettes (1-9, 10-19, or ≥ 20)), BMI (continuous).

d. Self-reported diabetes at recruitment and/or HbA1c ≥ 6.5% (48 mmol/mol).

e. Non-fasting = last intake < 3 hours ago, in between = last intake 3 - 6 hours ago, fasting = last intake ≥ 6 hours ago; p-interaction by fasting status based on Wald test: crude p = 0.905, BMI and smoking adjusted p = 0.806.