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Healthy lifestyle and the risk of pancreatic cancer in the EPIC study

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Abstract:	Background. Pancreatic cancer (PC) is a highly fatal cancer with currently limited	

	opportunities for early detection and effective treatment. Modifiable factors may offer pathways for primary prevention. In this study, the association between the healthy lifestyle index (HLI) and PC risk was examined. Methods and Findings. Within the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort, 1,113 incident PC (57% women) were diagnosed from 400,577 cancer-free participants followed-up for 15 years (median). HLI scores combined smoking, alcohol intake, dietary exposure, physical activity and, in turn, overall and central adiposity using BMI (HLIBMI) and waist-to-hip ratio (WHR, HLIWHR), respectively. High values of HLI indicate adherence to healthy behaviors. Cox proportional hazard models with age as primary time variable were used to estimate PC hazard ratios (HR) and 95% confidence intervals (CI). Sensitivity analyses were performed by excluding, in turn, each factor from the HLI score. Population attributable fractions (PAF) were estimated assuming participants' shift to healthier lifestyles. The HRs for a one-standard deviation increment of HLIBMI and HLIWHR were 0.84 (95% CI: 0.79, 0.89; ptrend=4.3e-09) and 0.77 (0.72, 0.82; ptrend=1.7e-15), respectively. Exclusions of smoking from HLIWHR resulted in HRs of 0.88 (0.82, 0.94; ptrend=4.9e-04). The overall PAF estimate was 19% (95% CI: 11%, 26%), and 14% (6%, 21%) when smoking was removed from the score. Conclusions. Adherence to a healthy lifestyle was inversely associated with PC risk, beyond the beneficial role of smoking avoidance. Public health measures targeting compliance with healthy lifestyles may have an impact on PC incidence.
Response to Reviewers:	Responses to the Reviewer's comments to the manuscript EJEP-D-19-00303 1) The authors have identified an interesting research question. "Healthy lifestyle and the risk of pancreatic cancer in the EPIC study" is an interesting topic. The main strengths of this study are that: 1) It uses a large study sample and addresses a clear and focused research question. 2) The overall study design is robust except for some minor aspects of details listed below 3) The exploration of the data using an extensive range of analysis is right. 4) The methods used are appropriate, and the presentation of the data is well performed. Some concerns which will need to be addressed are: The title is appropriate. Authors: We thank the Reviewer for highlighting the strengths of our manuscript. 2) Line 111-112: Please change sentence from "Public health measures targeting compliance to healthy lifestyles may have an impact on PC incidence." To "Public health measures targeting compliance with healthy lifestyles may have an impact on PC incidence." Authors: We updated the text according to the Reviewer's suggestion. 3) In the first paragraph please include the incidence and mortality rates for pancreatic cancer. Authors: The text was updated accordingly (page 5, lines 116-117) as: "Commonly diagnosed at late stages, PC is a highly fatal cancer with similar incidence and mortality rates [4]." 4) Line 123: please rewrite the sentence "United States and Western European countries, than in middle- and low-income countries" to "United States and Western European countries than in the middle and low-income countries". Authors: After double-checking, the original version of the sentence was kept. 5) In Exposure assessment: authors mention that "Habitual diet, including alcohol intake, over the year preceding recruitment was assessed at baseline by validated

	center-specific dietary questionnaires". Please explain how did the researchers exclude the memory bias of the participants? If not please add it to the limitations of the study. Authors: Following the Reviewer's suggestion, measurement error due to memory bias was mentioned in the discussion (page 14, lines 346-348), which now reads: "First, measurement errors likely affected dietary and lifestyle assessments, possibly introducing bias in estimated associations." 6) Line 238: please change sentence "regions (North: Denmark, Sweden; Central: United Kingdom, The Netherlands, Germany" to "regions (North: Denmark, Sweden; Central: the United Kingdom, The Netherlands, Germany" Authors: The text was updated accordingly. 7) Line 249: Reverse causation remains a concern despite excluding the first 2 and 5 years of follow-up. Could this issue be further mitigated by removal of participants that developed clinical disease over the first 10 years. Authors: In agreement with the Reviewer's request, further analyses were performed. Estimates were unchanged after removing the first 10 years of follow-up. 8) The statistical analyses are complete and well conducted Authors: We thank the Reviewer for this comment. 9) Line 317 after the line "Body fatness is also an established risk factor for PC [8]." Please also include the latest article https://www.ncbi.nlm.nih.gov/pubmed/30788774 Authors: The reference suggested by the Reviewer was added to the list of references. 10) Authors need to discuss in detail about the limitations of the study. Authors: In line with the Reviewer's request, further emphasis was given to the limitations of our study, other than mentioning measurement errors as a source of exposure misclassification. The text (page 14, lines 343-351) now reads: "However, this study also has limitations. First, measurement errors likely affected dietary and lifestyle assessments, possibly introducing bias in estimated associations. Furthermore, as EPIC participants represent a healthy proportion of the general population, risk estimates in our study were likely attenuated. In addition, the evidence for a role of life course socio-economic status on cancer-related risk factors was suggested [56], and the use of education in our study as a proxy for socio-economic status might have introduced residual confounding. Last, our study did not consider potential changes of dietary and lifestyle exposures after recruitment, which could be relevant to estimate accurately the association between lifestyle factors and PC risk, as well as to explain HR patterns over age." 11) Last paragraph can be made into a separate conclusion section. Authors: The text was updated accordingly. 12) Tables are appropriate. Overall a well conducted study. Authors: Thank you.

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Keywords

Pancreatic cancer; healthy lifestyle index; population attributable fraction; EPIC; prospective study.

Abbreviations

BMI: Body Mass Index

CI: Confidence Interval

EPIC: European Prospective Investigation into Cancer and Nutrition

HR: Hazard Ratio

PC: Pancreatic Cancer

PAF: Population Attributable Fraction

WCRF/AICR: World Cancer Research Fund/American Institute for Cancer Research

WHR: Waist-to-Hip ratio

Abstract (Words=248)

Background. Pancreatic cancer (PC) is a highly fatal cancer with currently limited opportunities for early detection and effective treatment. Modifiable factors may offer pathways for primary prevention. In this study, the association between the healthy lifestyle index (HLI) and PC risk was examined.

Methods. Within the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort, 1,113 incident PC (57% women) were diagnosed from 400,577 cancer-free participants followed-up for 15 years (median). HLI scores combined smoking, alcohol intake, dietary exposure, physical activity and, in turn, overall and central adiposity using BMI (HLI_{BMI}) and waist-to-hip ratio (WHR, HLI_{WHR}), respectively. High values of HLI indicate adherence to healthy behaviors. Cox proportional hazard models with age as primary time variable were used to estimate PC hazard ratios (HR) and 95% confidence intervals (CI). Sensitivity analyses were performed by excluding, in turn, each factor from the HLI score. Population attributable fractions (PAF) were estimated assuming participants' shift to healthier lifestyles.

Results. The HRs for a one-standard deviation increment of HLI_{BMI} and HLI_{WHR} were 0.84 (95% CI: 0.79, 0.89; p_{trend}=4.3e-09) and 0.77 (0.72, 0.82; p_{trend}=1.7e-15), respectively. Exclusions of smoking from HLI_{WHR} resulted in HRs of 0.88 (0.82, 0.94; p_{trend}=4.9e-04). The overall PAF estimate was 19% (95% CI: 11%, 26%), and 14% (6%, 21%) when smoking was removed from the score.

Conclusion. Adherence to a healthy lifestyle was inversely associated with PC risk, beyond the beneficial role of smoking avoidance. Public health measures targeting compliance with healthy lifestyles may have an impact on PC incidence.

Introduction (Words=4,134)

In the last decades, the rise in pancreatic cancer (PC) incidence has become a major public health concern with mortality rates expected to double by 2030 in American and European populations [1–3]. Commonly diagnosed at late stages, PC is a highly fatal cancer with similar incidence and mortality rates [4]. In the current absence of available screening tools [5], the identification of modifiable risk factors might be important for PC prevention.

The World Cancer Research Fund/American Institute for Cancer Research (WCRF/AICR) international expert panel estimated that at least one-third of all cancers could have been prevented through lifestyle management including diet, obesity and physical activity habits [6]. PC incidence rates are nearly four times higher in high-income countries such as the United States and Western European countries than in middle- and low-income countries [4], suggesting that PC occurrence may be associated with lifestyle factors specifically prevalent in the Western world. Individual examination of lifestyle risk factors of PC have led to the identification of smoking, as well as body fatness, adult attained height, type-2 diabetes, and heavy alcohol drinking as positive risk factors, while diet and physical activity have been inconsistently associated with PC risk [7,8]. There is limited evidence regarding the joint association of different lifestyle factors on PC incidence, especially among European populations [9,10].

Previous epidemiological studies have identified clusters of modifiable exposures, assessable through *a priori* scores reflecting compliance with primary prevention guidelines [11], which were evaluated in relation to cardiovascular diseases [12,13], cancer incidence [14,15], and overall and cause-specific mortality [16,17]. A multi-component score termed the Healthy Lifestyle Index (HLI), combining information on smoking, alcohol intake, dietary habits, body mass index (BMI), and physical activity has been previously related to colorectal [18], breast [19], gastric [20], and overall cancers [21] within the European Prospective

Investigation into Cancer and Nutrition (EPIC) study. Within the American Association of Retired Persons (AARP) study a strong inverse association was observed between the HLI and PC risk[9].

In this work, the association between the HLI and PC risk was examined within the EPIC study. Two versions of the score were used, i.e. (i) with BMI to reflect overall adiposity and (ii) with waist-to-hip ratio to reflect central adiposity. The marginal role of single factors in the HLI score was investigated, particularly smoking. Population attributable fractions were also estimated.

Material and Methods

Study population. EPIC is a multicenter prospective study designed to investigate the etiology of cancer in relation to diet and other lifestyle factors [22]. From 1992 to 2000, 521,324 participants aged from 35 to 70 years were recruited across 10 European countries, mostly from the general population, of which 70% were women. Exceptions were the French cohort (school and university employees), the Spanish and Italian centers (blood donors), Utrecht and Florence centers (breast cancer screening participants), and Oxford (vegetarians and 'health conscious' participants). In France, Utrecht and Naples women only were recruited. Study participants provided informed consent before completing questionnaires at baseline. Participants from Norway were excluded from this study, as information on physical activity was not compatible with the other centers [23].

Cancer cases were identified during follow-up based on population cancer registries in Denmark, Italy, Netherlands, Spain, Sweden, and the United Kingdom, and on a combination of methods, including health insurance records, contacts with cancer and pathology registries, and active follow-up of EPIC participants and their next of kin in France, Germany, and

Greece. Mortality data were collected from, either the cancer or mortality registries at the regional or national level.

The most recent vital status and cancer diagnosis update were used. Vital status was known for 98.4% of all EPIC subjects, while 1.6% of participants emigrated, withdrew or were lost to follow-up. The current follow-up period ended as follows: December 2009 in Varese and Murcia, December 2010 in Florence, Ragusa, Turin, Asturias, Bilthoven and Utrecht, December 2011 in Granada, Navarra, San Sebastian and Cambridge, December 2012 in Oxford, Umeå, and Denmark, and December 2013 in Malmö. The end of follow-up was considered as the last known contact with participants in France (June 2008), Heidelberg and Potsdam (December 2009), and Naples (December 2010) and Greece (December 2012). Cases of PC were primary incident tumor of the pancreas, coded according to the International Classification of Diseases (10^{th} edition), which included all invasive pancreatic cancers (C25.0–C25.3, C25.7–C25.9). Endocrine and neuroendocrine tumors of the pancreas (C25.4) were censored at date of diagnosis (n=54). Microscopically confirmed PC represented 83% of the cases (n=928) based on histology of the primary tumor or metastases, cytology or autopsy reports.

Exposure assessment. Habitual diet, including alcohol intake, over the year preceding recruitment was assessed at baseline by validated center-specific dietary questionnaires [22,24]. Data on anthropometry (self-reported in France and the UK Oxford center) [25,26] physical activity, smoking habits, and prevalent chronic conditions were collected at recruitment through lifestyle questionnaires [22].

A diet score was built from the combination of six dietary factors reflecting diet quality [21], i.e. cereal fibers, red and processed meat, the ratio of polyunsaturated to saturated fatty acids, margarine (to express industrially produced trans-fats) [27,28], glycemic load, and fruits and vegetables. For each dietary factor, residuals were computed in models with total energy

intake [29], and grouped into country-specific deciles. Individual scores were summed up and categorized into quintiles.

The HLI was generated from the combination of five lifestyle factors, namely: diet score, physical activity, smoking status, alcohol consumption and anthropometry. For each factor, scores ranging from 0 to 4 were assigned to increasingly healthier categories, as described in **Figure 1**. The HLI was obtained as the sum of scores of each lifestyle factor *[19]*. As previous evidence on PC etiology identified waist-to-hip ratio, an indicator of central adiposity, as a PC risk factor *[30,31]*, a HLI based on WHR (HLI_{WHR}) was implemented replacing BMI with sex-specific WHR quintiles.

Statistical analysis. From a study population of 521,324 participants, subjects without lifestyle or dietary information (n= 6,902), with ratio of estimated energy intake over energy requirement in the top or bottom 1% (n=10,241),*[32]* with self-reported prevalent cancer (n=24,221), with missing follow-up information (n=3,800), with missing smoking status (n=15,684) or physical activity (n=65,054) were excluded. For analyses with HLI_{WHR}, subjects with missing WHR were also excluded (n=45,105). Country-specific age standardized PC incidence rates (ASR, per 100,000 person-years, PY) were computed using 5-year categories in the range 50 to 70 years and the standard European population.

The association between the HLI and PC incidence was evaluated using multivariable Cox proportional hazard models, with age as the primary time variable, and Breslow's method to handle ties *[33]*. The time at study entry was age at recruitment, while the exit time was age at cancer diagnosis, death, loss, or end of follow-up, whichever came first. All models were stratified by study center *[32]*, sex and age at recruitment in 1-year categories.

The HLI_{BMI} and HLI_{WHR} were, in turn, modeled as continuous variables to compute HR estimates for a one-standard deviation (1-SD), corresponding to about three-point increase in the score. Analyses were also carried out in categories (0-4, 5-9, 10-14, 15-20), using the

group 5-9 as reference. Models were systematically adjusted for potential risk factors of PC and covariates influencing HLI and PC risk *[21,34–36]*, namely education level (no degree/primary school, secondary/technical or professional school, university degree or more, unknown (4%)), self-reported baseline diabetes status (no, yes, unknown (8%)), energy intake from non-alcohol sources (continuous), and height (continuous). Additional adjustment for BMI (continuous) was used in models for HLI_{WHR}. HRs were unchanged after women-specific inclusion of menopausal status, ever use of replacement hormonal replacement therapy and number of full-term pregnancies, thus adjustment for these variables was not pursued. Overall tests for statistical significance of HRs were determined by comparing Wald-test statistics to a χ^2 distribution with degree of freedom (dof) equal to the number of categories minus one for evaluation in categories (p_{Wald}) and dof equal to one as continuous (p_{trend}). The proportionality of hazards (PH) assumption was evaluated through the Schoenfeld's residuals *[37]*.

Sensitivity analyses were carried out by excluding, in turn, each factor from the HLI scores to identify factors mostly driving the HLI association with PC risk. The excluded component was used as a confounder in the model.

Assuming a causal relationship between HLI_{WHR} and PC risk, population attributable fractions (PAF) were estimated as the reduction in PC incidence that would occur if study participants shifted to the adjacent healthier category of HLI_{WHR}, as [38]

$$PAF = \frac{\sum_{i=1}^{k} RR_i \, c_i - \sum_{i=1}^{k} RR_i \, c_i^*}{\sum_{i=1}^{k} RR_i \, c_i},$$

with i=1,…,4 indexing the HLI_{WHR} categories, HR_i and c_i expressing the hazards ratio and the observed proportion of participants in category i, respectively, and c_i^* the counterfactual proportion of participants, as detailed in **Supplementary Table 1**. PAF was also computed assuming a counterfactual scenario whereby men adopted women's lifestyle habits. Given the

low PC prevalence and under the proportional hazards assumption, HRs were correct approximations of risk ratios (RR_i). Confidence intervals were obtained using bootstrap sampling [39].

The relationship between the HLI and PC risk was estimated by, in turn, sex, European regions (North: Denmark, Sweden; Central: The United Kingdom, The Netherlands, Germany; South: France, Greece, Italy, and Spain), and smoking status (never, former, current). Interactions were evaluated by comparing the difference in log-likelihood of models with and without interaction terms between HLI_{WHR} and, either sex, European region or smoking, to a χ^2 distribution, with dof equal to the total number of interaction terms minus one. Although the PH assumption was satisfied, possible selections could operate among study participants within 15 year of follow-up, and HR estimates can change with age. The pattern of HR for a 1-SD increase of HLI_{WHR} by age was examined using a flexible parametric survival model on the cumulative hazard scale. Restricted cubic splines with 5 internal knots were used to model the baseline hazard using attained age as the time scale and a time-varying coefficient on HLI_{WHR} [40].

To address potential reverse causality, analyses were carried out excluding the first 2 and 5 years of follow-up. In analyses excluding smoking from the HLI, HR estimates after adjustment by smoking status (never, former, current), smoking intensity (number of cigarette/day, continuous) and duration of smoking (years, continuous) were examined. Two-sided p-values were used with a 5% nominal statistical significance. Analyses were performed using Stata 14 [41].

Results

From a total of 400,577 participants (70% women) followed-up for 15 years (median) and a total of 5,544,627 person-years, 1,113 incident PC cases were diagnosed. Exclusion of subjects without information on their WHR led to 1,075 PC cases from a total of 355,472 participants as reported in **Table 1**. The overall PC ASR was equal to 6.0 per 100,000 person-years, with relatively large and low ASR estimates observed in Germany (9.4 per 100,000 PY) and France (2.1 per 100,000 person-years), respectively. The individual components of the HLI, together with other confounding variables, are described in **Table 2**. The HLI was inversely related to education, while the prevalence of diabetes at recruitment was stable across HLI categories. The hypothesis of PH assumption was not rejected with p-value equal to 0.24.

A 1-SD higher HLI was inversely associated with PC risk, with HR equal to 0.84 (95%CI: 0.79, 0.89, p_{trend}=4.3e-09) for HLI_{BMI} and 0.77 (0.72, 0.82, p_{trend}=1.7e-15) for HLI_{WHR}, as shown in **Table 3**. These patterns were confirmed for PC HR estimates for analyses in categories, consistently for HLI_{BMI} and HLI_{WHR}.

Results of sensitivity analyses are displayed in **Figure 2**. After exclusion of smoking status, the HR for a 1-SD increase of HLI_{BMI} was 0.94 (95%CI: 0.88, 1.01; p_{trend}=0.11), and after exclusions of, in turn, alcohol and BMI, HRs were 0.85 (0.80, 0.91; p_{trend}=6.3e-07) and 0.79 (0.74, 0.85; p_{trend}=7.6e-12), respectively. After exclusion of, in turn, smoking, alcohol, waist-to-hip ratio from the HLI_{WHR} score, HRs were equal to 0.88 (0.82, 0.94; p_{trend}=4.9e-04), 0.79 (0.74, 0.84; p_{trend}=7.0e-13) and 0.79 (0.74, 0.85; p_{trend}=3.2e-11), respectively.

PAF estimates for a shift of participants to the adjacent healthier category of HLI_{WHR} was equal to 19% (95%CI: 11%, 26%) (**Table 4**). Excluding, in turn, smoking, alcohol and WHR from the HLI_{WHR} showed PAF estimates of 14% (6%, 21%), 19% (10%, 25%), and 16% (9%, 22%), respectively. PAF were 8% (-3%, 18%) for non-smokers at baseline (never and former) and 20% (7%, 35%) for current smokers. PAF estimates were 29% (16%, 37%) in

men, and 13% (2%, 24%) in women. Counterfactual scenario whereby men adopted women's lifestyle habits showed a PAF of 13% (9%, 26%).

The association between the HLI_{WHR} and PC risk were similar by sex, European region, and smoking status with $p_{heterogeneity}$ equal to 0.35, 0.15 and 0.62, respectively (**Figure 3**). Although the PH assumption was satisfied, PC HR estimates for HLI_{WHR} showed weaker associations at older ages (**Figure 4**). Exclusion of the first 2 and 5 years of follow-up did not materially alter HRs. After exclusion of smoking from the HLI and adjustment by smoking status, intensity and duration, HRs were unchanged (not shown).

Discussion

In this large European prospective study, healthy lifestyle habits expressed as a HLI score were strongly inversely related to the risk of PC. Adherence to healthy behaviors corresponding to a three-point increase in the score was associated with a 16% (95%CI: 11%, 21%) lower PC risk for a score that included BMI, and 23% (18%, 28%) lower PC risk for a score based on WHR. These results support the adoption of healthy lifestyles in PC prevention.

Scores reflecting dietary and lifestyle habits have become increasingly popular in cancer epidemiology research [21,42,43]. In EPIC, scores expressing adherence to either the Mediterranean diet or the WCRF/AICR recommendations have mainly focused on diet, physical activity and anthropometry, and had previously shown null associations with PC risk in both men and women [44,45]. Within the NIH-AARP study, a score based on the American Cancer Society recommendations including physical activity, diet, BMI, alcohol, but not smoking, was associated with a 20% (95%CI: 3%, 35%) lower PC risk in men, comparing the top vs. bottom category, while no association was observed in women [46].

Within the same cohort, an inverse association was observed between HLI and PC, when smoking was added to the score [9].

In the current study, a comprehensive evaluation of the association between HLI and PC risk was undertaken using sensitivity analyses. As smoking is an established strong risk factor of PC [47], it has been suggested that the association between lifestyle habits and PC might be primarily driven by smoking [45]. In our analysis, HLI was inversely associated with PC risk even after excluding smoking from the score, with a 12% risk reduction associated with a three-point (1-SD) increase in the HLI_{WHR} (95%CI: 6%, 18%; p_{trend}=4.9e-04). Additionally, in never and former smokers, the PC HR for a three-point increase in the HLI was equal to 0.87 (0.79, 0.95; p_{trend}=2.0e-03, data not shown), suggesting the advantage of adopting healthy habits for PC prevention, beyond the benefit of smoking avoidance.

Body fatness is also an established risk factor for PC [8,48]. A recent pooled analysis concluded that central adiposity during adulthood assessed through waist circumference, or waist-to-hip ratio may also predict PC risk independently from BMI [49]. In our study, HLI based on WHR showed a marginally stronger relationship with PC risk than HLI based on BMI. The subcutaneous truncal adipose tissue has been positively associated with the development of insulin resistance and diabetes [31,50,51], two recognized risk factors for PC [52], and may explain the role of central adiposity, rather than overall adiposity, in PC etiology. Moreover, smoking and alcohol consumption have been previously associated with increasing visceral fat deposition [53,54], which may suggest common pathways between smoking, alcohol consumption and central adiposity in pancreas carcinogenesis.

In our study, the association between HLI and PC was marginally stronger at younger ages compared to older ages. This pattern could be due to a depletion overtime of participants susceptible to PC [55], a phenomenon resulting in an over representation of non-susceptible participants with adverse lifestyle profiles at older ages, thus leading to weaker relationships.

Alternatively, HR patterns could be ascribed to study participants' changes towards healthier lifestyle habits related to ageing, or ultimately due to a true causal association indicating that PC benefits could be more substantial if favorable lifestyle habits were adopted at younger ages [56].

This study is to date the first evaluation of the association between a combination of healthy lifestyle factors and PC incidence in European populations, thus corroborating previous evidence from a US study [9]. The strengths of the present study rely on its prospective multi-country design reflecting heterogeneous lifestyle habits. Its large sample size and long follow-up time allowed ascertainment of over a thousand incident PC cases, increasing the statistical power in comparison with the previous EPIC evaluation [44]. Furthermore, associations were unchanged after exclusion of the first years of follow-up. However, this study also has limitations. First, measurement errors likely affected dietary and lifestyle assessments, possibly introducing bias in estimated associations. Furthermore, as EPIC participants represent a healthy proportion of the general population, risk estimates in our study were likely attenuated. In addition, the evidence for a role of life course socio-economic status on cancer-related risk factors was suggested [57], and the use of education in our study as a proxy for socio-economic status might have introduced residual confounding. Last, our study did not consider potential changes in dietary and lifestyle exposures after recruitment, which could be relevant to estimate the association between lifestyle factors and PC risk, as well as to explain HR patterns over age.

Assuming that HLI was causally related to PC risk, and that combinations of different lifestyle factors leading to the same value of the HLI had the same effect on PC risk, PAF estimates indicated that 14% (95%CI: 6%, 21%) of PC could have been avoided by controlling central adiposity, alcohol consumption, diet and physical activity, and up to 19% (11%, 26%) if smoking control was also implemented, indicating the benefit of adopting

healthy lifestyle beyond smoking control. In the AARP study, the PAF was 27% assuming that participants adopted the healthiest lifestyle pattern [9], while in a recent Australian PC study considering only smoking and BMI, the PAF was 30% [58].

Conclusion

In conclusion, our findings provide evidence that adherence to a combination of healthy lifestyle habits was strongly inversely associated with PC risk in European adults. Inverse associations were observed even after dismissing, in turn, smoking, alcohol drinking, and adiposity. Adherence to healthy lifestyle habits, especially from younger ages, could be an effective primary prevention strategy to control the incidence of PC, a fatal cancer with no screening tools currently available for early detection.

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Conflict of interest

None to declare.

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Data sharing statement

Information to submit an application to have access to EPIC data and/or biospecimens can be found at http://epic.iarc.fr/access/index.ph.

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Figures Captions

Fig 1 Scoring system implemented to combine the 5 lifestyle factors into the Heathy Lifestyle Index based on the waist-to-hip ratio (HLI_{WHR})

[1] For the HLI_{BMI}, sex-specific waist-to-hip ratio quintiles was replaced by categories of BMI at baseline using cut-offs as (4) 22–23.9 $kg.m^{-2}$, (3) 24–25.9$kg.m^{-2}$, (2) <22 $kg.m^{-2}$, (1) 26–29.9$kg.m^{-2}$, and (0) >30 $kg.m^{-2}$.

Fig 2 Hazard ratio estimates for the associations between a 1-SD increment of HLI[1] and PC risk after recalculation of the HLI_{BMI} and the HLI_{WHR} excluding, in turn, each lifestyle factor

[1] One Standard deviation corresponded to about 3 units of either HLI_{BMI} or HLI_{WHR};

[2] Models evaluating associations between the HLI_{BMI} and PC risk were adjusted for education level, diabetes status, non-alcohol energy intakes, height, and the index components currently excluded from the calculation of the HLI, and stratified by study center, age and sex;

[3] Models evaluating associations between the HLI_{WHR} and PC risk were adjusted for education level, diabetes status, non-alcohol energy intakes, height, BMI and the index components currently excluded from the calculation of the HLI, and stratified by study center, age and sex.

Fig 3 Heterogeneity in the relationship between HLI_{WHR} and PC by sex, European region, and smoking status, expressed for a 1-SD increase of HLI_{WHR}[1]

[1] One Standard deviation corresponded to about 3 units of either HLI_{BMI} or HLI_{WHR};

[2] Northern Europe included Denmark and Sweden, Central Europe included United Kingdom, The Netherlands and Germany, and Southern Europe included France, Greece, Italy and Spain;

[3] Models were computed using the HLI_{WHR} excluding smoking;

[4] Models included interaction terms between HLI_{WHR} and, in turn, sex, European region, and smoking status at recruitment. Differences in HRs were assessed comparing the log-likelihood of models with and without interaction terms to a χ^2 distribution with degrees of freedom equal to the number of categories minus one.

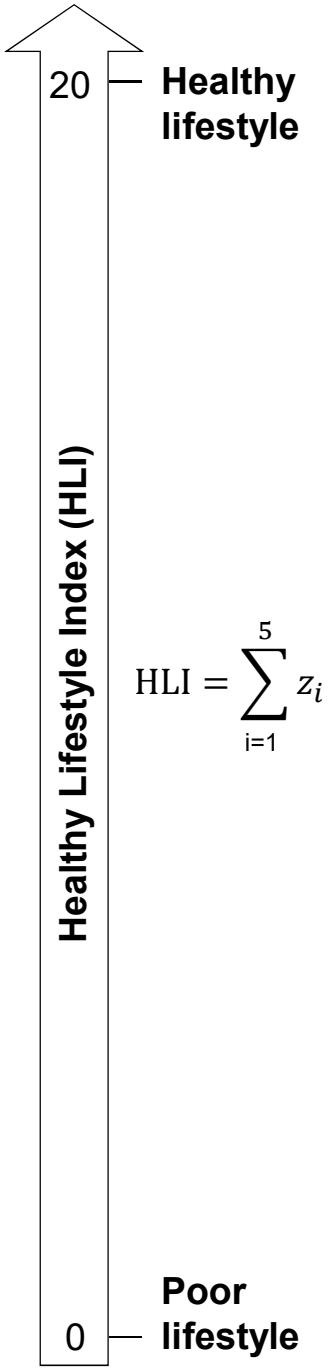
Fig 4 Hazard ratio function (and 95%CI)[1] for the association between HLI_{WHR} and PC risk over years of age, for 1-SD increase of HLI_{WHR}

[1] Obtained from a flexible parametric survival model using restricted cubic splines with 5 internal knots and a time-varying coefficient on HLI_{WHR}. Model was adjusted for educational level, BMI, height, non-alcohol energy intake, diabetes status, sex, country, age at recruitment.

Figure 1

Click here to access/download;Figure;Fig1.pdf

Modifiable lifestyle factors (z_i)	Levels of exposure	Level Score
Smoking status	Never	4
	Ex-smokers > 10 years	3
	Ex-smokers ≤ 10 years	2
	Current ≤15 cig/day	1
	Current > 15 cig/day	0
Alcohol intake (in g/day)	< 6	4
	6 - 11.9	3
	12 - 23.9	2
	24 - 59.9	1
	≥ 60	0
Waist-to-Hip ratio[1]	1st quintile (M: <89 ; W: <73)	4
	2nd quintile (M: 0.90-0.92 ; W: 0.74-0.77)	3
	3rd quintile (M: 0.93-0.95 ; W: 0.78-0.80)	2
	4th quintile (M: 0.96-0.99 ; W: 0.81-0.85)	1
	5th quintile (M: >1.00 ; W : >0.86)	0
Diet score Cereal fiber, red and processed meat, ratio of polyunsaturated to saturated fat, margarine, glycemic load, and fruits and vegetables	5th quintile (34-54)	4
	4th quintile (30-33)	3
	3rd quintile (26-29)	2
	2nd quintile (22-25)	1
	1st quintile (0-21)	0
Physical activity (in MET-hours/week)	5th quintile (M: >96 ; W : >134)	4
	4th quintile (M: 67-96 ; W: 96-134)	3
	3rd quintile (M: 47-67 ; W: 69-96)	2
	2nd quintile (M: 30-47 ; W: 45-69)	1
	1st quintile (M: <30 ; W: <45)	0



$$\mathrm{HLI} = \sum_{i=1}^{5} z_i$$

Figure 2

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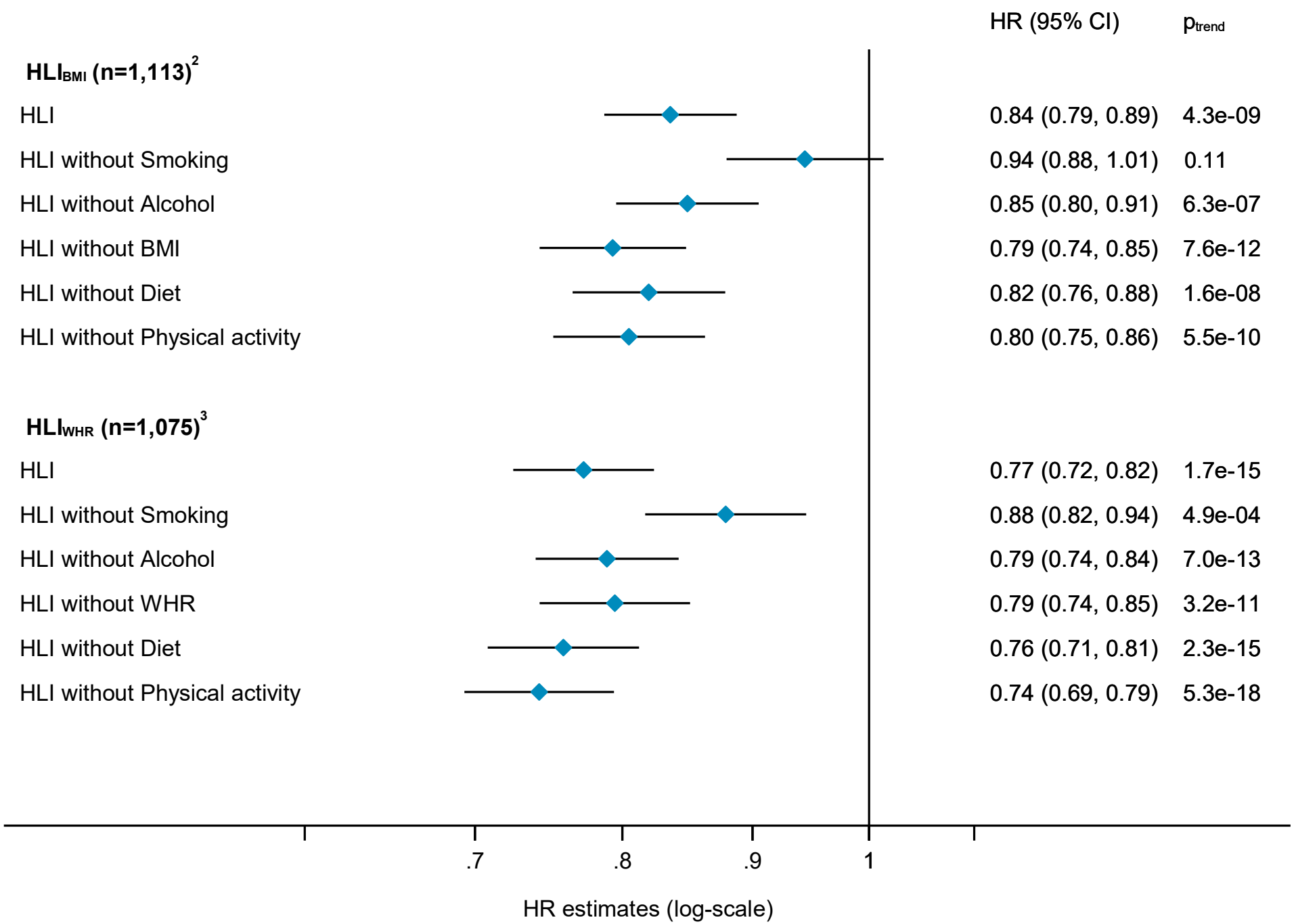


Figure 3

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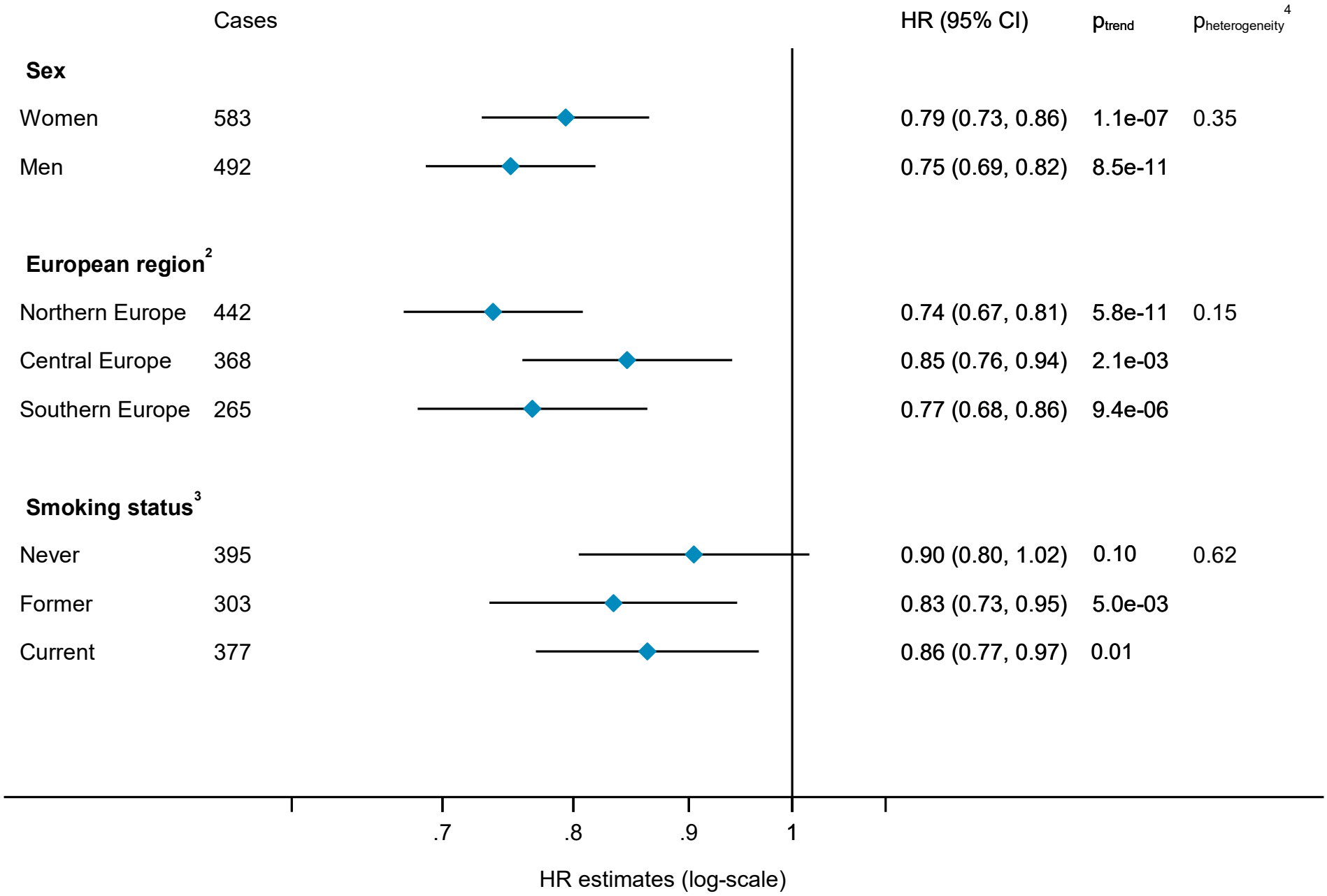


Figure 4

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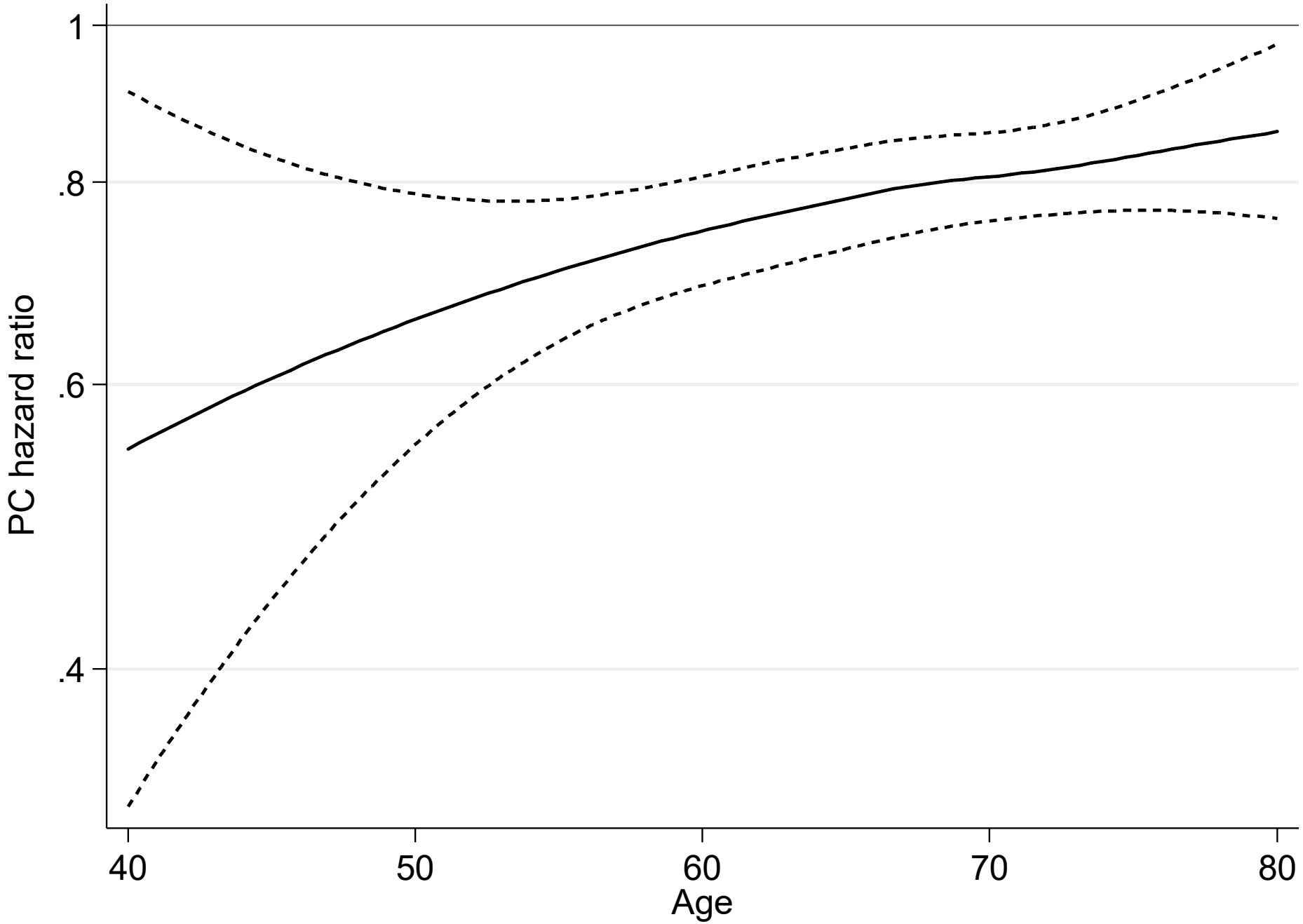


Table1 Click here to access/download;table;Table1.docx

Table 1 Country-specific distribution of study participants, PC cases and HLI_{WHR} score in the EPIC cohort

	Participants	PC cases	FUP[1] (years)	PY	ASR[2]	Age at baseline[1] (years)	HLI_{WHR}[1]	
Denmark	53,570	314	16 (5-18)	794,475	8.5	57	10	(4-15)
France	18,973	16	15 (6-15)	252,504	2.1	53	11	(6-16)
Germany	48,001	115	12 (4-14)	498,390	9.4	51	12	(6-17)
Greece	24,686	41	11 (3-16)	266,330	3.8	53	12	(6-17)
Italy	44,263	103	15 (7-17)	626,927	6.0	51	12	(6-17)
Spain	39,852	105	17 (9-19)	635,724	5.8	49	13	(6-17)
Sweden	24,038	128	18 (4-22)	394,001	7.4	58	11	(6-16)
NL	30,550	81	15 (6-17)	429,979	5.8	50	13	(7-17)
UK	71,539	172	16 (5-18)	1,069,797	5.2	49	13	(8-18)
All participants	355,472	1,075	15 (5-18)	4,968,127	6.0	52	12	(7-17)

Abbreviations: ASR, age-standardized incidence rate; FUP, follow-up; HLI, healthy lifestyle index; NL: The Netherlands; PC, pancreatic cancer; PY, person-years; UK: United Kingdoms;

[1] Median values (5^{th} and 95^{th} percentiles);

[2] Country-specific age-standardized incidence rates (per 100,000) were computed using 5-year categories in the age band 50 to 70 years and the standard European population.

Click here to access/download;table;Table2.docx

Table 2 Baseline characteristics[1] of the EPIC participants by categories of the waist-to-hip ratio-based healthy lifestyle index (HLI_{WHR})

	All	HLI_{WHR} categories			
		[0 - 4]	[5 - 9]	[10 - 14]	[15 - 20]
Number of participants *(n)*	355,472	6,594	79,446	189,732	79,700
Number of PC cases	1,075	45	355	515	160
Person-Years	4,968,127	86,277	1,085,850	2,647,497	1,148,503
Smoking *(% never)*	45	0	13	48	78
Alcohol *(g/day)*	7 (0-48)	36 (7-84)	16 (0-53)	6 (0-30)	2 (0-13)
BMI *(kg/m^2)*	25 (20-33)	27 (21-35)	26 (20-34)	25 (20-34)	24 (19-33)
Waist-to-hip ratio	0.83 (0.72, 1.01)	0.98 (0.81-1.08)	0.91 (0.75-1.04)	0.83 (0.71-0.99)	0.77 (0.69-0.92)
Diet score *(units)*	27 (19-39)	21 (13-29)	24 (15-35)	27 (17-38)	32 (22-41)
Physical activity *(MET-hours/week)*	74 (19-154)	29 (5-73)	47 (10-129)	78 (23-175)	118 (50-205)
Age at recruitment *(years)*	52 (39-64)	54 (41-64)	53 (37-66)	53 (34-68)	51 (30-67)
Height *(cm)*	165 (152-182)	171 (157-184)	168 (154-183)	165 (152-182)	164 (151-181)
Weight *(kg)*	68 (51-95)	80 (58-106)	75 (54-101)	70 (52-95)	66 (51-88)
Diabetes *(%)*	3	3	3	3	3
Education *(% below secondary)*	31	45	37	33	30
Energy from non-alcohol drinking *(kcal/day)*	1,959 (1,337-2,814)	2,304 (1,430-3,675)	2,158 (1,311-3,433)	2,011 (1,229-3,209)	1,966 (1,211-3,095)

[1] Medians (5^{th} - 95^{th} percentiles) are presented for continuous variables, percentages for categorical variables.

Click here to access/download;table;Table3.docx

Table 3 Hazard Ratio estimates for associations between the combined HLI_{BMI} and HLI_{WHR} and PC risk in the EPIC study

	HLI_{BMI}[2]					HLI_{WHR}[3]				
	Cases	PY	HR	(95%CI)	p-value[4]	Cases	PY	HR	(95%CI)	p-value[4]
HLI Categories										
[0 - 4]	33	78,738	1.49	(1.03 - 2.13)		45	86,277	1.45	(1.06 - 1.99)	
[5 - 9]	338	1,271,983	1.00	Ref.		355	1,085,850	1.00	Ref.	
[10 - 14]	570	3,061,318	0.75	(0.65 - 0.87)		515	2,647,497	0.65	(0.57 - 0.75)	
[15 - 20]	172	1,132,588	0.64	(0.52 - 0.78)	3.0e-07	160	1,148,503	0.55	(0.45 - 0.68)	6.2e-13
1-SD increase[1]										
	1,113	5,544,627	0.84	(0.79 - 0.89)	4.3e-09	1,075	4,968,127	0.77	(0.72 - 0.82)	1.7e-15

[1] One standard deviation corresponded to about 3 units of either HLI_{BMI} or HLI_{WHR};

[2] Models were adjusted for education level, diabetes status, and non-alcohol energy intakes, height, and stratified by study center, age in 1-year category, and sex;

[3] Models were adjusted for education level, diabetes status, non-alcohol energy intakes, height, BMI and stratified by study center, age in 1-year category, and sex;

[4] For categorical variables, p-values were determined using a Wald test for overall significance, according to a χ^2 distribution with degrees of freedom equal to the number of categories minus one.

Table4 Click here to access/download;table;Table4.docx

Table 4 Population attributable fractions (PAF) of PC assuming counterfactual scenarios with the HLI_{WHR} in the EPIC study

	PAF (%)	(95%CI)
Shift to the adjacent healthier category of HLI_{WHR}		
All participants	19%	(11%, 26%)
without Smoking	14%	(6%, 21%)
without Alcohol	19%	(10%, 25%)
without WHR	16%	(9%, 22%)
without Diet	21%	(15%, 26%)
without Physical activity	17%	(9%, 23%)
Non-smokers	8%	(-3%, 18%)
Smokers	20%	(7%, 35%)
Men	29%	(16%, 37%)
Women	13%	(2%, 24%)
Shift of lifestyle habits[2]		
Men to Women	13%	(9%, 16%)

[1] Confidence intervals were obtained using a bootstrap sampling procedure using 1,000 iterations;

[2] The counterfactual proportions of men across HLI_{WHR} categories were equal to the observed proportions of women in each HLI_{WHR} categories.

Cover letter revision
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14 August 2019

Dear Professor Hofman,

We thank you and the Reviewer for the valuable feedback to our manuscript “Healthy lifestyle and the risk of pancreatic cancer in the EPIC study” (EJEP-D-19-00303).

We carefully revised the manuscript according to the Reviewer’s comments, which contributed to further improve the text. You will find it enclosed, together with our point-by-point response letter.

In addition, the notation used to estimate population attributable fractions was further clarified in the methods (page 9, line 228-234), in the discussion (page 14, lines 354-356), and in Supplementary Table 1. All changes were highlighted in the text. Copyright statements were also added.

Thank you in advance for considering this revised version of the manuscript. We were glad to hear that the manuscript was accepted for publication by EJEP, and we look forward to hearing from you about the next steps of the publication process.

Yours sincerely,

Pietro Ferrari
Head, Nutritional Methodology and Biostatistics Group
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Rebutal
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Responses to the Reviewer's comments to the manuscript *EJEP-D-19-00303*

1) The authors have identified an interesting research question. "Healthy lifestyle and the risk of pancreatic cancer in the EPIC study" is an interesting topic. The main strengths of this study are that:

1) It uses a large study sample and addresses a clear and focused research question.

2) The overall study design is robust except for some minor aspects of details listed below

3) The exploration of the data using an extensive range of analysis is right.

4) The methods used are appropriate, and the presentation of the data is well performed.

Some concerns which will need to be addressed are:

The title is appropriate.

Authors: *We thank the Reviewer for highlighting the strengths of our manuscript.*

2) Line 111-112: Please change sentence from "Public health measures targeting compliance to healthy lifestyles may have an impact on PC incidence." To "Public health measures targeting compliance with healthy lifestyles may have an impact on PC incidence."

Authors: *We updated the text according to the Reviewer's suggestion.*

3) In the first paragraph please include the incidence and mortality rates for pancreatic cancer.

Authors: *The text was updated accordingly (page 5, lines 116-117) as: "Commonly diagnosed at late stages, PC is a highly fatal cancer* ***with similar incidence and mortality rates*** *[4]."*

4) Line 123: please rewrite the sentence "United States and Western European countries, than in middle- and low-income countries" to "United States and Western European countries than in the middle and low-income countries".

Authors: *After double-checking, the original version of the sentence was kept.*

5) In Exposure assessment: authors mention that "Habitual diet, including alcohol intake, over the year preceding recruitment was assessed at baseline by validated center-specific dietary questionnaires". Please explain how did the researchers exclude the memory bias of the participants? If not please add it to the limitations of the study.

Authors: *Following the Reviewer's suggestion, measurement error due to memory bias was mentioned in the discussion (page 14, lines 346-348), which now reads: "**First,** measurement errors likely affected **dietary and lifestyle assessments, possibly introducing bias in estimated associations.**"*

6) Line 238: please change sentence "regions (North: Denmark, Sweden; Central: United Kingdom, The Netherlands, Germany" to "regions (North: Denmark, Sweden; Central: the United Kingdom, The Netherlands, Germany"
Authors: *The text was updated accordingly.*

7) Line 249: Reverse causation remains a concern despite excluding the first 2 and 5 years of follow-up. Could this issue be further mitigated by removal of participants that developed clinical disease over the first 10 years.
Authors: *In agreement with the Reviewer's request, further analyses were performed. Estimates were unchanged after removing the first 10 years of follow-up.*

8) The statistical analyses are complete and well conducted
Authors: *We thank the Reviewer for this comment.*

9) Line 317 after the line "Body fatness is also an established risk factor for PC [8]." Please also include the latest article https://www.ncbi.nlm.nih.gov/pubmed/30788774
Authors: *The reference suggested by the Reviewer was added to the list of references.*

10) Authors need to discuss in detail about the limitations of the study.
Authors: *In line with the Reviewer's request, further emphasis was given to the limitations of our study, other than mentioning measurement errors as a source of exposure misclassification. The text (page 14, lines 343-351) now reads: "However, this study **also** has limitations. **First,** measurement errors likely affected **dietary and lifestyle assessments, possibly introducing bias in estimated associations. Furthermore, as EPIC participants represent a healthy proportion of the general population, risk estimates in our study were likely attenuated. In addition,** the evidence for a role of life course socio-economic status on cancer-related risk factors was suggested [56], and the use of education **in our study** as a proxy for socio-economic status might have introduced residual confounding. **Last**, our study did not consider potential changes of **dietary and** lifestyle exposures after recruitment, which*

could be relevant ***to estimate accurately the association between lifestyle factors and PC risk, as well as*** *to explain HR patterns over age."*

11) Last paragraph can be made into a separate conclusion section.
Authors: *The text was updated accordingly.*

12) Tables are appropriate. Overall a well conducted study.
Authors: *Thank you.*

Cover letter
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Ref.: NMB/PF/kz/19/07 05 April 2019

Dear Dr Hofman,

Enclosed please find a manuscript entitled "**Healthy Lifestyle and the risk of pancreatic cancer in the EPIC study**", which we would like to submit for publication to the European Journal of Epidemiology. Our study provided a comprehensive evaluation of the impact of modifiable lifestyle factors on the risk of pancreatic cancer (PC), a highly fatal disease with limited opportunities for early detection and effective treatment.

Within the European Prospective Investigation on Cancer and Nutrition (EPIC) study, the risk of PC was evaluated with respect to the Healthy Lifestyle Index (HLI), a score combining information on smoking history, alcohol intake, a dietary score, obesity and physical activity. Based on 1,113 incident cases, a strong inverse association between HLI and PC risk was observed, with a 16% lower PC risk (95% CI: 11%, 21%) for one standard deviation increase in the HLI score, expressing adherence to healthier lifestyle habits. Sensitivity analyses showed consistent inverse associations in scores where smoking, alcohol and obesity were, in turn, excluded. Using flexible parametric modelling to appreciate trends over participants' age, the inverse association between HLI and PC risk was marginally stronger among adults younger than 55 years than at older ages.

Population attributable fractions based on counterfactual scenarios were estimated for a shift of participants towards healthier lifestyle habits. Our findings suggest that controlling modifiable factors like obesity, alcohol consumption, adopting a healthy diet and increasing the level of physical activity could prevent 14% (95% CI: 6%, 21%) of PC cases, and up to 19% (11%, 26%), if quitting smoking was also considered. Interestingly, 13% (9%, 16%) of PC cases could be prevented if men adopted lifestyle habits of women. Overall, our findings suggest that public health measures targeting compliance to healthy lifestyles may have a strong impact to control PC incidence.

We confirm that the manuscript has been submitted solely to the European Journal of epidemiology, and that it has not been previously published, either in whole or in part, nor have the findings been posted online. We confirm that all Authors have approved the manuscript for submission. We have no conflicts of interest to disclose.

Thank you in advance for your consideration of this manuscript.

Yours sincerely,

Pietro Ferrari, PhD
Head, Nutritional Methodology and Biostatistics Group (NMB)

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Article title Healthy Lifestyle and the risk of Pancreatic cancer in the EPIC study

Manuscript No. (if you know it) EJEP-D-19-00303

Author name Sabine NAUDIN

Are you the corresponding author? ☐ Yes ☒ No

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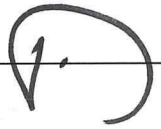
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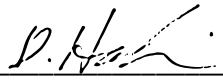
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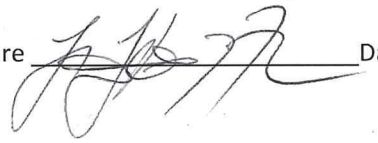
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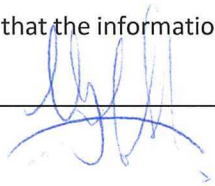
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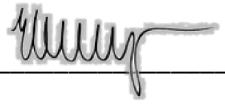
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Manuscript No. (if you know it) EJEP-D-19-00303

Author name Flavie PERRIER

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Category of disclosure	Description of Interest/Arrangement

Article title Healthy Lifestyle and the risk of Pancreatic cancer in the EPIC study

Manuscript No. (if you know it) EJEP-D-19-00303

Author name Fiona MCKENZIE

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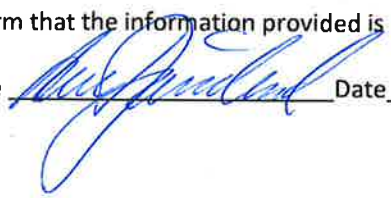
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Author name Verena Katzke

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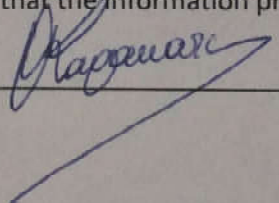
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Author name VALERIA PALA

Are you the corresponding author? ☐ Yes ☒ No

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Category of disclosure	Description of Interest/Arrangement

Article title Healthy Lifestyle and the risk of Pancreatic cancer in the EPIC study

Manuscript No. (if you know it) EJEP-D-19-00303

Author name GIOVANNA MASALA

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Manuscript No. (if you know it) EJEP-D-19-00303

Author name CARLOTTA SACERDOTE

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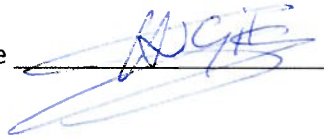
Article title Healthy Lifestyle and the risk of Pancreatic cancer in the EPIC study

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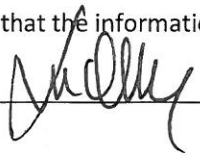
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Author name ISABEL DRAKE

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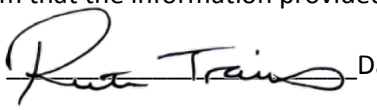
Article title Healthy Lifestyle and the risk of Pancreatic cancer in the EPIC study

Manuscript No. (if you know it) EJEP-D-19-00303

Author name Ruth Travis

Are you the corresponding author? ☐ Yes ☐ No
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Article title Healthy Lifestyle and the risk of Pancreatic cancer in the EPIC study

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Author name Eric Duell

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Healthy Lifestyle and the risk of Pancreatic cancer in the EPIC study

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Author name Paul Brennan

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