Higher Meat Intakes Are Positively Associated with Higher Risks of Developing Pancreatic Cancer in an Age-dependent Manner and Are Modified by Plasma Anti-oxidants: A Prospective Cohort Study (EPIC-Norfolk) Using Data from Food Diaries

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ABSTRACT

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Objective: Carcinogens in meat may be involved in pancreatic carcinogenesis. Meat intake was investigated using 7-day food diaries (7-DFDs) and according to factors potentially influencing carcinogenesis: age, cooking method and anti-oxidants.

Methods: Twenty-three thousand one hundred and thirty-three participants in the EPIC-Norfolk cohort study completed 7-DFDs and were followed up. Meat intakes were compared with controls and hazard ratios (HR) calculated.

Results: Eight-six participants developed pancreatic cancer. If younger than 60 years at recruitment, all quintiles of red meat (Q1 vs Q5, HR, 4.62, 95% Confidence Interval (CI), 0.96-22.30, *P* = 0.06) and processed meat (Q1 vs Q5, HR, 3.73, 95% CI, 0.95-14.66, *P* = 0.06) were non-significantly positively associated, with significant trends across quintiles (HR_{trend}, 1.33, 95% CI, 1.01-1.77, and HR_{trend}, 1.37, 95% CI, 1.04-1.82 respectively). Red meat's effect was attenuated by higher, but not lower plasma vitamin C (HR, 1.06, 95% CI, 0.69-1.63 vs HR, 1.84, 95% CI, 1.09-3.14), and for processed meat (HR, 1.07, 95% CI, 0.71-1.63 vs HR, 1.80, 95% CI, 1.10-2.96). A non-statistically significant risk was observed for high temperature cooking methods in younger people (HR, 4.68, 95% CI, 0.63–34.70, *P* = 0.13).

Conclusions: Red and processed meats may be involved in pancreatic carcinogenesis.

Key words: Pancreatic cancer; diet; epidemiology.

Introduction

Pancreatic cancer causes 227,000 deaths worldwide annually and is the eighth commonest cause of cancer related deaths[1]. The cancer has the worst prognosis of any malignancy[2], with only 16% of patients surviving one year and just 3% to five years[3]. The incidence of pancreatic cancer has a significant geographical variation, which supports an aetiological role for lifestyle factors, including diet[1]. A high meat intake may be involved, as cooking meat at *higher temperatures* produces potentially carcinogenic heterocyclic amines (HCAs) and polycyclic aromatic hydrocarbons (PAHs). HCAs induce molecular changes primarily through oxidation, resulting in the formation of DNA adducts[4], which are associated with the development of pancreatic ductal carcinomas in hamsters[5]. PAHs may exert a carcinogenic effect through forming epoxides which react with DNA to induce genetic mutations[6]. When administered to rats, the PAH dimethylbenzanthracene, induces pancreatic ductal adenocarcinoma that is histologically similar to that in humans[7,8]. Furthermore, meat preservation results in the formation of nitrosamines which promote tumour growth in animal models of pancreatic cancer including inducing mutations in the K-*ras*, *p53, p16 and DPC-4* genes[9].

The plausible mechanisms for how meat intake may promote pancreatic carcinogenesis have not consistently been supported by data from observational epidemiological studies. The Continuous Update Project (CUP) from the World Cancer Research Fund (WCRF) concluded the evidence was inconsistent[10] and included a meta-analysis of 11 prospective observational studies of 6,643 cancer cases[11]. Here there was no association between red meat consumption of > than 1 portion size (120g) per day and the relative risk of pancreatic cancer (RR=1.13, 95% CI=0.93-1.39). A positive association with eating one portion (50g) per day of processed meat was associated with a higher risk (RR= 1.19, 95% CI = 1.04–1.36).

Limitations of this analysis were: statistical inconsistency between the results of investigations ($P_{heterogeneity}$=0.001), a lack of generalisability as in five studies just one gender was studied and important covariates including diabetes and body mass index were not always considered. In all studies there was measurement error for recording habitual meat intake, as food frequency questionnaires (FFQs) documented meat consumption, which are less accurate than 7-day food diaries (7-DFDs), which also record detail on food preparation methods[12]. The effect of white meat was not reported in this meta-analysis. Further observational studies are required using detailed measurements of dietary intake to provide clarification. Importantly, we are not aware of any previous observational work investigating dietary meat intake at younger ages, which may be when carcinogenesis is initiated as the epithelium may then be more susceptible to mutagens.

The aim of this study was to address the limitations of previous work by conducting a prospective cohort study using dietary information derived, for the first time from(7-DFDs, in both genders and adjusted for covariates. Additionally, we conducted specific dietary analyses to support several biological mechanisms for meat in pancreatic carcinogenesis, namely: age at intake, high temperature cooking which may produce a higher carcinogen load and vitamin C bioavailability which may inhibit the potential deleterious pro-oxidant effects of meat carcinogens. Meat intake at different ages may be important, as pancreatic carcinogenesis may have a long natural history, progressing firstly through the formation in younger people of pre-malignant lesions, namely pancreatic intra-epithelial neoplasia (PanINs) and consequently their malignant transformation. PanINs are initially non-invasive microscopic epithelial neoplasms which probably require up to 10 years to progress to malignancy[13,14,15,16]. Further mechanistic information would be provided by demonstrating attenuation of any positive associations of meat by a higher bioavailability of anti-oxidants

thereby supporting the hypothesis of the pro-oxidant carcinogenic meat effect[17]. Demonstrating associations, with precision, would support measuring age-dependent meat intakes in aetiological studies and offer a potential preventative strategy to reduce the incidence of this highly aggressive cancer.

Materials and Methods

The cohort was 23 133 men and women aged between 39 – 79 years, recruited into the European Prospective Investigation of Cancer-Norfolk (EPIC-Norfolk) study between the years 1993 and 1997. All participants were resident in the county of Norfolk, UK and were registered in one of 35 primary care general practices. The recruitment questionnaires recorded participants': demography, previous medical history, regular medications, smoking status and habitual diet; the latter in a 7-DFD. Participants underwent a baseline health check by a nurse, which included taking a non-fasting blood sample and anthropometric measurements[18]. The blood samples were transported to the laboratory for analyses, including plasma vitamin C, a measure of intake and bioavailability. The nurse explained the 7-DFD, the first day of which was a 24 hour recall of their previous day's dietary intake. The remaining 6 days were recorded by the participants themselves at home, who documented their complete daily dietary intake including: all food types, portion sizes, brands, home cooking methods and recipes at eight separate meal and snack times each day. Portions of certain foods were quantified in household measures or by weight, with photographs supplied to aid their estimation of portion sizes. The completed 7-DFDs were subsequently returned to the research centre where they were then coded by trained data-entry staff using a specially designed computer programme called DINER (Data Into Nutrients for Epidemiological Research)[19]. Here, each diary entry was matched to the closest description

of one of 11 000 food items and 55 000 portion sizes. This software facilitated the translation of participant-reported free text of their dietary intake to structured food and nutritional data. The DINER nutrient database is based on foods in the United Kingdom Food Composition Database, compiled by the Royal Society of Chemistry and food manufacturer's databases. Each 7-DFD took approximately 1-2 hours to code with an average of 220 individual food and drink items reported by participants in their diaries. From this process the daily intakes of disaggregated red, processed and white meat in grams per day were calculated. This measure represents the actual amount of meat consumed, since only the meat quantity in meat containing dishes counted towards meat intake[20]. Data was checked using DINERMO, a program designed for data cleaning and checking[20]. The Norwich District Health Authority Ethics Committee granted approval for the study and all participants gave their signed consent for future review of their medical records.

Following recruitment, the cohort was monitored to identify those participants who subsequently developed pancreatic cancer up to June 2010. These cases were identified by matching the EPIC-Norfolk database with the hospital admissions records of both Norfolk Health Authority and the Eastern Cancer Registry and Information Centre. A medical gastroenterologist verified each cancer diagnosis through reviewing the clinical notes for both the appropriate symptomatology and confirmatory investigations. The exclusion criteria were: diagnostic uncertainty for cases, and pancreatic cancer diagnosed before, or within 12 months of study entry. The latter helped ensure that the nutritional information was truly prospective before the development of symptoms.

The analysis was a case-cohort design, comparing meat intakes between participants who developed pancreatic cancer with a random sample of 3970 participants without pancreatic cancer. The baseline characteristics were compared between cases and controls using a t-

test for normally distributed continuous variables, a Mann-Whitney U test for non-parametric continuous variables and a Chi-squared test for categorical ones. The dietary intakes of: red, processed and white meats were divided into quintiles across the distribution of the whole cohort. Cox proportional hazard regression models estimated the hazard ratios (HRs), and 95% confidence intervals (CIs), for each quintile with the risk of pancreatic cancer, with the lowest quintile as the reference category. The first model was adjusted for the covariates of age at recruitment and gender. A second model included these variables plus: smoking status (never, previous or current smoker), diabetes mellitus (yes or no) and total energy intake (quintiles). A third model added dietary antioxidant intake (vitamin C, vitamin E and selenium – analysed as a binary variable for intake) and physical activity (four levels of intensity), both of which we have previously reported to be inversely associated with risk in this cohort[21,22]. Finally, these models were adjusted for standard categories of body mass index (BMI) to ascertain if any effects of meats were not reduced which would suggest meat acts mechanistically via increasing BMI rather than components of the meat itself. The hazard ratios for trends across quintiles for all models were calculated. The percentage of cases of pancreatic cancer associated with the four highest quintiles of meat intakes were calculated for any with positive associations. The formula used was that for the population attributable fraction (summation of(HR-1/HR in each quintile) x (% of cases in each of the 4 higher quintiles))[23].

To provide data on possible carcinogenic mechanisms of meat we conducted 3 other analyses. Firstly, we compared the meat intakes of participants recruited younger and older than 60 years, with this age cut-off being the average age of participants at recruitment (mean=59.5 years, median=59.4 years). Demonstrating associations in younger people may suggest meat acts at the earlier stages of carcinogenesis possibly by inducing PanINs.

Secondly, total meat intakes were analysed according to cooking methods as *higher temperatures* produce more potential carcinogens. A category of total meat intake cooked at *higher temperatures* was generated, consisting of one or more of the 5 cooking methods: grilled, barbequed, baked, fried and roasting. The reference category ('no meat eaten cooked at *higher temperatures*') included meat cooked by other methods: boiled, stewed, braised, casseroled and microwaved. If participants did not record the cooking method for meat, an estimate where possible of this was made by the diary coder based on the type of food recorded. This cooking method analysis was performed in participants younger and older than 60 years at recruitment to determine if the effects were age-dependent. Thirdly, the trends for hazard ratios across quintiles of meat intakes were calculated in participants with vitamin C plasma levels firstly above and secondly below the median vitamin C concentration, to investigate if there was effect modification of meat intake according to the bioavailability of this antioxidant. Effect modification by plasma vitamin C, an anti-oxidant, would support a pro-oxidant mechanism for meat.

Results

In the 23 133 participants, 86 (0.37%) developed pancreatic cancer (56% women, median age at diagnosis 73.4 years) between 1 and 17 years after recruitment (table 1) at a median time interval of 8.9 years (1.1 – 15.3 years). The median ages at diagnosis were 63.1 years in those recruited younger than 60 years and 77.7 years in those older than 60 years at recruitment. The medical notes confirmed all cases had the appropriate symptoms and at least one confirmatory diagnostic radiological imaging modality. Most cases (80.2%) had cancer extending outside the pancreas (i.e. at least American Joint Committee on Cancer (AJCC) stage III) and diagnostic histology was available for 35%. Overall, the median survival

of cancer cases was 4 months (IQR 2-7 months). Cases with and without histology were similar, both for median survival (3 vs 4 months, p=0.73) and metastases (78% vs 73%, p=0.65). Only 8% were treated surgically, 35% received chemotherapy and most (57%) were treated palliatively. Characteristics of the random sample of 3970 controls were similar to those of the whole EPIC-Norfolk cohort (median recruitment age 59.3 vs 59.2 years, men 44% vs 46%, respectively). Cases were approximately 6 years older than controls at recruitment ($p < 0.001$), although there were no significant differences in either: gender, diabetes mellitus, smoking status, or median intakes of energy and the 3 meat types (table 1). Plasma vitamin C data was available for 85% of cases and 88% of controls.

In the multivariable analyses in the whole cohort, there were no significant associations between the intakes of either red or processed meats with pancreatic cancer risk in any of the quintiles, or for any trends across quintiles (table 2). For white meat, all quintiles were positively associated which were statistically significant for quintiles 2 and 4, with a more than a doubling of the risk of pancreatic cancer, but with no trends across quintiles. All the estimates were of similar magnitude in all three statistical models. However, in participants younger than 60 years at recruitment for red meat intake there was a non-statistically significant positive association for all higher quintiles (1^{st} vs 5^{th} quintile HR=4.62, 95% CI=0.96–22.30, p=0.06) and a biological gradient across quintiles (HR_{trend}=1.33, 95% CI=1.01–1.77, $p<0.05$), with similar findings for processed meat (1^{st} vs 5^{th} quintile HR=3.73, 95% CI=0.95–14.66, HR_{trend}=1.37, 95% CI=1.04–1.82, $p<0.05$) (table 3). In participants older than 60 years at recruitment, red meat was inversely associated with risk in each quintile, but there was no biological gradient. In this older age group for processed meat, there were no associations in any quintile or for trends. For white meat, there were no associations in participants younger than 60 years of age, but for older ones, all quintiles were associated

with at least a doubling of the risk, which was statistically significant only in the 2nd and 4th quintiles, but with no trends across quintiles. The effect sizes were similar when other covariates were included in all these age-dependent models. When including BMI, the higher significant hazard ratios for quintiles and for trends across quintiles in meat intakes in participants younger than 60 years were similar. The population attributable fraction for red and processed meat intakes in participants younger than 60 years at recruitment were 18% and 16%, respectively for all pancreatic cases. In post hoc analyses, performed as positive associations existed for both red and processed meats in participants younger than 60 years, the trends across quintiles for each meat type, when adjusted for the other, were HR_{trend}=1.32, (95% CI=0.99-1.77) and HR_{trend}=1.36, (95% CI=1.02-1.81) respectively, which were similar to when not adjusting. When the intake of both meats were summed there was a positive trend across quintiles of total intake (HR trend =1.48, 95% CI 1.10-1.99, P=0.01). To provide supportive information on possible biological carcinogenic mechanisms for meat intake two further analyses were conducted. Firstly, for temperature-dependent cooking methods, there was a higher risk for total meat cooked at *higher temperatures* limited to those younger than 60 years at recruitment, but was not statistically significant (HR=4.68, 95% CI=0.63-34.70, p=0.13) (table 4). Secondly, there was effect modification, by plasma vitamin C concentrations, for both red and processed meats in those younger than 60 years. In those with levels within the lowest half of plasma vitamin C, there was still a statistically significant higher risk of pancreatic cancer for both red meat (trend across quintiles HR=1.84, 95% CI=1.09–3.14, p<0.05) and processed meat (trend across quintiles HR=1.80, 95% CI=1.10-2.96) (table 5), but none for these two meats in participants with the highest 50% of vitamin C. There were no associations with vitamin C concentrations in those older than 60 years.

Discussion

The main findings of this aetiological study were statistically significant positive associations with higher intakes of both red and processed meats for developing pancreatic cancer in participants younger, but not older than 60 years, at recruitment. Evidence that this may be a causal association includes: plausible biological mechanisms including pro-oxidants in meat inducing genetic changes, large effect sizes and biological gradients across intakes. The prospective reporting of meat consumption and adjusting for potential confounders ensured the findings had both temporality and analogy. The effect sizes were similar in all models, including when considering BMI, suggesting meat has an independent effect on risk. Interventional randomised trials of either increased intake or avoidance of meat in the general population would be unethical and logistically impractical to confirm our findings and only observational studies can investigate meat intake in pancreatic cancer.

Our data provides some evidence for possible mechanisms of meat in pancreatic carcinogenesis, which may be related to: younger ages at intake, higher temperature cooking methods and low antioxidant consumption. Firstly, the positive associations with red and processed meats in younger, but not older participants suggest that possible carcinogens may influence the earlier stages of pancreatic carcinogenesis, possibly through the formation and progression to malignancy of PanINs. These lesions are recognised microscopic precursors of pancreatic cancer, divided into three grades dependent on their degree of epithelial atypia[15,24]. The prevalence increases with age and they are commoner in the pancreatic head where pancreatic cancers are also more frequent[14]. Genetic analyses suggest more than a decade may elapse between the first genetic 'hit' of pancreatic ductal adenocarcinoma and invasive cancer, with a further 6 years before metastases developed[16].

Hypothetically, as the median age at diagnosis of pancreatic cancer in the Norfolk cohort was 73 years, and if the evolution of cancer is slow, carcinogens in red meat could be pathogenic in those younger than 60 years, which is supported by our data. The reasons no consistent positive associations with red and processed meats in participants older than 60 years were seen are unknown, although possibly the pancreatic ductal epithelium may be less susceptible to carcinogens then. Another possibility is that older participants who eat large amounts of meats may have other dietary habits which protect against carcinogenesis. Possibly, they may consume more dietary antioxidants, as previously we reported in this cohort that higher intakes of vitamin C, vitamin E and selenium were associated with lower risks of pancreatic cancer[21]. The associations we documented with white meat were less consistent than those for other meats. For white meat, in the whole cohort, and participants older than 60 years, we documented positive associations in some but not all quintiles, although there was no biological gradient which is against a causal association. Further follow-up of the cohort is required to identify further cases and allow more precise effect sizes to be estimated.

The second possible mechanism for meat and carcinogenesis that our findings support is the attenuated effects of red and processed meats in participants with a higher bioavailability of vitamin C. As the positive association with red meat in younger people was nullified by higher plasma antioxidant vitamin C levels, this supports an aetiological role of HCA acting through pro-oxidation. Furthermore, the positive associations persisted in those with the lowest vitamin C levels. Thirdly, cooking red meat at high temperatures produces carcinogenic HCAs and PAHs[25-27]. Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), the most abundant HCA in meat forms DNA adducts in the pancreas in animal models, possibly through pro-oxidation[28]. We documented a nearly 5 times greater non-significant

risk of pancreatic cancer in those younger than 60 years at recruitment cooking meat at *higher temperatures*. This may be an under-estimate of any effect size, as if participants did not record the cooking method an estimate was made where possible by the diary coders based on the recorded food eaten which could introduce measurement error.

To give more precise estimates of any effect, further cohort follow up is now required to acquire more cases. Previously, only a few case-control studies, and no cohort investigations, have investigated meat cooking methods or HCA/PAH intakes. A US retrospective study of 193 cases reported that grilled or barbecued red meat intake was positively associated with pancreatic cancer (odds ratio (OR)=2.19 (95% CI=1.40-3.40)) [29]. Another US retrospective study documented positive associations with the calculated dietary intake of HCAs and a PAH, with the following ORs: PhIP 1.80 (95% CI 1.00-3.10), DiMeIQx 2.00 (95% CI 1.20-3.50) and Benzo(a)pyrene (B(a)P) 2.2 (95% CI 1.20-4.00)[27]. The authors assigned HCA and B(a)P contents values to meat items listed on the dietary questionnaire. They used a recognised technique for screening genotoxic HCAs in cooked foods, using solid-phase extraction and high performance liquid chromatography with ultraviolet light and fluorescence detection[30]. Therefore, the experimental and epidemiological evidence suggests cooking meat at high temperatures may be carcinogenic.

For processed meat a biological mechanism for carcinogenesis may be through forming mutagenic N-nitroso compounds[31]. Such meats are preserved with nitrites which form N-nitroso compounds with chemicals in meat. They are also generated endogenously in the stomach from dietary nitrite and ingested amides in animal foods[32]. These reach the pancreas via the bloodstream where they may induce pancreatic cancer as demonstrated in animal models[9,33]. A US prospective cohort study of 303,156 participants reported that men with the highest quintile of estimated summed nitrate or nitrite intake from processed meat

had a small non-significantly elevated risk of pancreatic cancer (HR=1.18, 95% CI 0.95–1.47, P-trend=0.11)[34]. More epidemiological studies are required which assess the intake of N-nitroso containing meats to look for consistency. Finally, as the positive associations we reported with red and processed meats were independent of BMI, this suggests meat effects are not related to mechanisms linked to adiposity.

This study design has several methodological strengths and weaknesses. A strength was the 7-DFD which improved the precision of the estimates for meat intake[35]. Using 16-day weighed records as a standard for measuring diet, the correlation coefficient with 7-DFDs for protein intake was 0.66 compared to from FFQs of only 0.43[12]. The prospective design reduced both recall bias for diet, and selection bias as both future cases and controls were drawn from the same population. Follow-up bias will be low as cases were identified from a comprehensive cancer registry and there is geographical cohort stability, with 94.6% participants having local postcodes 20 years after recruitment. Covariates for pancreatic cancer were considered, including red and processed meats, which did not alter the magnitude of the associations suggesting the latter two operate through different biological mechanisms. The results are generalisable as our cases were similar to those expected including: incidence, demography, cancer stage, treatments and survival[36]. In our investigation only 35% of cases had histological confirmation of pancreatic cancer, which was due to the relative unavailability of diagnostic techniques to obtain pancreatic tissue in the time when cases were diagnosed (1993–2010). However, the medical notes of potential cases were reviewed to ensure cases had both the appropriate symptoms and confirmatory investigations. The total meat intake in participants was similar to national data in the United Kingdom suggesting our results are generalizable. The median total meat intake for participants over 65 years in our study was 87g/day (men) and 67g/day (women), compared

to national averages in these ages of 78g/day and 55g/day respectively[37]. There is potential measurement error for habitual diet by analysing just one assessment at recruitment, as dietary habits may vary over time. However, as this error applies to both cases and controls then the magnitude of any true effect size would be falsely underestimated, rather than spuriously over-estimated. The meat intake in the UK has varied little over the study period, although change from more red meat to poultry was reported over 17 years in 5 362 participants[38]. There may be residual confounding for meat as other components in meat such as iron and fat, which may be the true aetiological factor in meat responsible for pancreatic carcinogenesis[39]. Finally, the positive associations we reported have large confidence intervals due to small numbers in some groups, so further cohort follow up is required to acquire more cases to increase precision of these estimates.

Existing epidemiological studies have not consistently reported positive associations with meat intake possibly as most have not measured consumption at either: specific ages, according to anti-oxidant bioavailability or cooking methods. For example, a prospective study of 477,202 participants in the whole EPIC cohort across Europe (of which EPIC-Norfolk is part) reported no associations between red or processed meats and pancreatic cancer, although diet was measured by FFQs, not 7-DFDs[40]. Similarly, other cohort studies have not reported age-dependent effects or investigated cooking methods[11]. The time period of the study is important as nitrite and nitrate levels have altered. Nitrite concentrations have decreased, whereas nitrate levels have either increased or decreased according to foods consumed[34].

In conclusion, our findings support the hypothesis that potential carcinogens in red and processed meats may be involved in pancreatic carcinogenesis, possibly acting in younger people. These could influence the development of precursor lesions for pancreatic cancer.

The potential biological mechanisms, large effect sizes, dose-response relationships and the absence of associations with higher vitamin C levels are supportive of a role for meat. Meat intakes should be recorded in future epidemiological studies and assessed according to: age, cooking methods and vitamin C. If further observational studies confirm our findings then reducing the population's meat intake may contribute to lowering the incidence of this highly aggressive cancer.

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Table 1: Baseline characteristics of participants

		Controls	Cases
Number		3970	86
Age at recruitment (median, IQR, years)		59.3 (39.8 – 77.9)	65.5 (45.9 – 76.7)*
Females	**(n, %)**	2230 (56.2%)	48 (55.8%)
Males	**(n, %)**	1740 (43.8%)	38 (44.2%)
Diabetes Mellitus	**(yes, n, %)**	121 (3.0%)	4 (4.7%)
Current smoker	**(n, %)**	451 (11.4%)	8 (9.3%)
Previous smoker	**(n, %)**	1670 (42.4%)	39 (45.3%)
Never smoked	**(n, %)**	1818 (46.2%)	39 (45.3%)
Total energy intake (calories/day, median, IQR)		1882 (1578 – 2259)	1812 (1494 – 2205)
BMI (kg/m^2)			
<25	**(number, %)**	1571 (39.6%)	41 (47.7%)
25-30	**(number, %)**	1779 (44.9%)	36 (41.9%)
30-35	**(number, %)**	502 (12.7%)	7 (8.1%)
>35	**(number, %)**	110 (2.8%)	2 (2.3%)
Physical activity			
Inactive	**(n, %)**	1206 (30.4%)	32 (37.2%)
Moderately inactive	**(n, %)**	1146 (28.9%)	20 (23.3%)
Moderately active	**(n, %)**	895 (22.5%)	20 (23.3%)
Active	**(n, %)**	723 (18.2%)	14 (16.2%)
Dietary anti-oxidant intake			
High	**(number, %)**	3879 (97.7%)	79 (91.9%)
Low	**(number, %)**	91 (2.3%)	7* (8.1%)
Red meat intake	**(g/day, median, IQR)**	29.9 (12.4 – 49.7)	31.8 (9.0 – 50.4)
Processed meat intake	**(g/day, median, IQR)**	18.7 (7.5 – 32.0)	19.1 (9.0 – 35.0)
White meat intake	**(g/day, median, IQR)**	19.4 (4.1 – 36.7)	22.1 (8.9 – 39.0)
Cooking methods (n, %)			
Higher temperatures		3455 (87.0%)	79 (91.9%)
Lower temperatures		515 (13.0%)	7 (8.1%)
Plasma	**Lower** 3-54 mmol/L	91 (2.3%)	7 (8.1%)
	Higher > 54 mmol/L	3879 (97.7%)	79* (91.9%)

IQR = Inter-quartile range, *P < 0.005 between cases and controls.

Table 2: Meat intakes and the risk of developing pancreatic cancer in the whole cohort

Red meat (quintiles and cut points (g/day))	Controls (n)	Cases (n)	HR[1] (95% CI)	HR[2] (95% CI)	HR[3] (95% CI)
0 - < 8.6	793	19	1.00	1.00	1.00
8.6 – < 23.1	795	16	0.74 (0.39 – 1.44)	0.75 (0.39 – 1.44)	0.77 (0.39 – 1.50)
23.1 - < 36.8	799	12	0.54 (0.26 – 1.10)	0.54 (0.26 – 1.10)	0.55 (0.26 -1.13)
36.8 - < 55.7	785	26	1.14 (0.63 – 2.05)	1.13 (0.63 – 2.05)	1.15 (0.63 – 2.10)
55.7 – 349.3	798	13	0.59 (0.29 – 1.19)	0.60 (0.29 – 1.19)	0.62 (0.30 – 1.29)
Processed meat (quintiles and cut points (g/day))					
0 - < 5.4	800	13	1.00	1.00	1.00
5.4 – < 14.2	790	20	1.36 (0.67 - 2.74)	1.38 (0.68 - 2.78)	1.43 (0.71 – 2.90)
14.2 - < 23.1	794	17	1.16 (0.56 - 2.38)	1.18 (0.57 - 2.44)	1.27 (0.61 – 2.64)
23.1 – < 36.1	795	17	1.20 (0.58 - 2.47)	1.22 (0.59 - 2.54)	1.31 (0.63 – 2.72)
36.1 – 192.0	791	19	1.42 (0.69 - 2.91)	1.45 (0.70 - 3.00)	1.57 (0.75 – 3.27)
White meat (quintiles and cut points (g/day))					
0 - < 0.2	905	12	1.00	1.00	1.00
0.2 – < 14.3	683	23	2.45 (1.22 - 4.93)	2.44 (1.12 - 4.91)	2.46 (1.22 – 4.95)
14.3 - < 25.3	803	11	1.03 (0.45 – 2.34)	1.04 (0.46 - 2.37)	1.08 (0.48 – 2.47)
25.3 - < 41.4	782	26	2.47 (1.25 – 4.90)	2.47 (1.25 - 4.91)	2.54 (1.28 – 5.05)
41.4 – 347.8	797	14	1.45 (0.67 – 3.15)	1.48 (0.68 - 3.20)	1.58 (0.73 – 3.44)

HR[1] Adjusted for age at recruitment and sex.
HR[2] Model 1 plus: smoking, diabetes mellitus and total energy intake.
HR[3] Model 2 plus: antioxidant intake and physical activity.

Table 3: Meat intakes and the risk of pancreatic cancer stratified by age at recruitment

Red meat (quintiles and cut off points (g/day))	Controls		Cases		Age <60 HR (95% CI)	Age ≥60 HR (95% CI)
	n^1	n^2	n^1	n^2		
0 - < 8.6	445	347	2	17	1.00	1.00
8.6 – < 23.1	400	395	6	10	3.23 (0.65 – 16.12)	0.51 (0.23 – 1.12)
23.1 - < 36.8	401	399	4	8	2.13 (0.39 – 11.73)	0.38 (0.16 – 0.89)
36.8 - <55.7	386	399	8	18	4.32 (0.91 – 20.59)	0.80 (0.41 – 1.57)
55.7 – 349.3	435	363	8	5	4.62 * (0.96 – 22.30)	0.24 (0.08 – 0.67)
Processed meat (quintiles and cut off points (g/day))						
0 - < 5.4	476	324	3	10	1.00	1.00
5.4 – < 14.2	387	403	4	16	1.56 (0.35 – 7.01)	1.21 (0.55 – 2.68)
14.2 - < 23.1	396	398	6	11	2.64 (0.66 – 10.62)	0.81 (0.34 – 1.92)
23.1 – < 36.1	385	410	7	10	3.35 (0.86 – 13.09)	0.75 (0.31 – 1.83)
36.1 – 192.0	429	362	8	11	3.73* (0.95 – 14.66)	0.90 (0.37 – 2.17)
White meat (quintiles and cut off points (g/day))						
0 - < 0.2	470	435	6	6	1.00	1.00
0.2 – < 14.3	336	347	7	16	1.60 (0.53 – 4.81)	3.40 (1.32 – 8.77)
14.3 - < 25.3	414	389	0	11	N/A	2.19 (0.80 – 5.96)
25.3 - < 41.4	390	392	10	16	1.94 (0.70 – 5.38)	3.05 (1.19 – 7.82)
41.4 – 347.8	457	340	5	9	0.96 (0.29 – 3.16)	2.06 (0.73 – 5.81)

n^1 Participants <60 years at recruitment, n^2 Participants ≥60 years at recruitment

* Hazard ratio trend across quintiles P-value <0.05

All models were adjusted for: age at recruitment, sex, smoking, diabetes mellitus and total energy intake.

Table 4: Meat cooking temperature and the risk of developing pancreatic cancer

Meats eaten cooked at higher temperatures[1]	Controls	Cases	Whole cohort (95% CI)	Age < 60 (95% CI)	Age ≥ 60 (95% CI)
NO	515	7	1.00	1.00	1.00
YES	3455	79	1.49 (0.69 – 3.25)	4.68* (0.63 – 34.70)	1.02 (0.44 – 2. 38)

[1] Grilled, barbequed, baked, fried or roasted
*p=0.13

Table 5: The effects of plasma vitamin C on meat intake and risk of developing pancreatic cancer in those younger than 60 years at recruitment

Plasma Vitamin C	Controls	Cases	Red meat HR_{trend} (95% CI)	Processed meat HR_{trend} (95% CI)	White meat HR_{trend} (95% CI)
Lower half (3-54 mmol/L)	899	12	1.84* (1.09–3.14)	1.80* (1.10-2.96)	0.80 (0.54-1.20)
Higher half (>**54–170 mmol/L)**	925	12	1.06 (0.69-1.63)	1.07 (0.71-1.63)	1.33 (0.88-2.02)

HR_{trend} = Hazard Ratio across quintiles of meat intake