Original Article

Major Dietary Protein Sources in Relation to Pancreatic Cancer: a Large Prospective Study

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Abstract

Introduction: Current evidence is inconsistent about the association between dietary protein intake and risk of pancreatic cancer (PC). The aim of this study was to evaluate the association between total intake of major dietary protein sources and risk of PC in a large prospective study in Golestan Cohort Study (GCS).

Methods: We examined the association of total intake of major dietary protein sources with risk of PC in the 50,045 participants (20,855 men and 28,255 women) of the GCS in northeastern Iran. Participants were aged 40 and older at baseline and actively followed from 2004 to the present time. Dietary data were collected using a validated semi-quantitative food-frequency questionnaire that was administered at baseline. Cox proportional hazards models were used to estimate Multivariable hazard ratios with 95% confidence intervals (CI).

Results: During 383,630 person-years of follow-up, 54 cases of pancreatic cancer were ascertained. There was only a statistically significant inverse association between risk of PC for the second versus lowest tertile of plant based protein intake in the first and multivariable models (HR95% ,27. = CI = .12–.64, HR = .28, 95% CI = .12 –.65 respectively); however, this association was not significant anymore when comparing the highest tertile with the lowest one, in the first and multivariable models (HR = .49, 95% CI = .19–1.24, HR = .52, 95% CI = .20–1.34 respectively).

Conclusion: In this large prospective cohort, we did not observe any clear and consistent evidence for an association between main dietary protein intakes and the risk of PC. Examining the method of cooking is recommended in future studies.

Keywords: Dietary protein, pancreatic neoplasm, Prospective Cohort Study, red meat

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Introduction

Pancreatic cancer (PC) is ranked as the 7th leading cause of cancer death worldwide with over 330,000 deaths per year and it has had the least improvement among other cancers.[1,2]

As a result of PC's poor prognosis and the absence of well-established risk factors, the most effective strategy for diminishing the incidence of this cancer is primary prevention which includes understanding the etiology of this fatal malignancy and in particular, identifying its modifiable risk factors.[1,3,4] Body mass index (BMI), adiposity, excess body weight and obesity,[2,5–8] diabetes mellitus and raised level of HbA1c,[2,6,7,9,10] genetic susceptibility, and a family history of cancer (specially PC),[6,8,10,11] smoking,[2,6–8] alcohol abuse,[2,7] and chronic pancreatitis[6,7] are known risk factors for PC.

In Western countries, several case control and prospective studies have evaluated the association between different types of dietary protein sources such as red and processed meat, chicken and poultry, dairy, egg, legumes and nut in relation to PC risk; however, the results are controversial and inconclusive. Most of case control studies have shown a positive relationship between red and processed meat and PC risk,[12–15] while few prospective studies have reported this association,[16–19] and most prospective studies have shown no significant association.[20–24] Results of meta-analysis on case-control and prospective studies which have evaluated total, red or processed meat, poultry, dairy products and fish in relation to PC are still inconsistent and need further prospective investigations to completely elucidate the role of these dietary factors in etiology of PC.[25,26]

There is no study evaluating the association between different dietary protein sources (e.g., animal or plant based protein) and PC risk in the Middle East region with its special dietary habits. Thus, we designed this study to investigate the association of total intake of major dietary protein sources and in particular, intake of different types of dietary proteins in relation to the PC in a large prospective study in Iran.

Materials and Methods

Study population

The Golestan Cohort Study (GCS) is an ongoing large prospective population based cohort study, which was launched in 2004 in Golestan Province, in northeastern Iran, by recruiting 50,045 adults, aged between 40 and 87 years, from Gonbad city and 326 rural villages (a 20% urban, 80% rural cohort).[27–29]

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The cohort study was initially planned to explore the causes of esophageal cancer (EC) in the Golestan region as a high risk area for this malignancy. The details of the study's profile, design and features have been explained elsewhere.[29,30]

At baseline, the participants were interviewed by a trained interviewer to complete a comprehensive questionnaire, which included a validated semi-quantitative food frequency questionnaire,[31] a questionnaire about history of opium and tobacco using,[32] cigarette smoking and alcohol drinking, as well as medical history of diagnosed diseases and medications, and a general questionnaire, which supplied details of individual's lifestyle and socio-demographic characteristics. Anthropometric measurements (such as weight, height, waist and hip circumferences) were taken by trained research staff.

After exclusion of cases who were affected by prevalent cancers (except non-melanoma skin cancer) at baseline (n = 147) based on the local population-based cancer registry, in addition to excluding subjects with missing or incomplete information on FFQ (dietary) and/or potential confounders' data (e.g., demographic, socio-economic, history of diabetes, smoking, alcohol and opium using, anthropometric and educational status) (n = 932), we further excluded under- and over-report of energy intakes (e.g. Less than 500 or more than 5000 kcal/d) (n = 182), and unreasonable BMI range (less than 15 or more than 50 kg/m^2) (n = 108). The final cohort population included in the analysis was 48676 subjects (20,683 men and 27,993 women) with 54 pancreatic cancer cases.

At the time of the enrollment, participants were asked to sign a written informed consent. The study ethical approval was gained from the Institutional Review Board of the Digestive Disease Research Institute (DDRI) of Tehran University of Medical Sciences (Tehran, Iran).

Dietary assessment

A semi-quantitative 116-item FFQ was designed particularly for the population of northeastern Iran based on their typically consumed foods and preferences to assess their usual dietary intake during the previous year. Each subject was interviewed by a trained nutritionist to complete the FFQ, which was previously validated at the pilot phase of the GCS. To assess the validity and reliability of this FFQ, for one year, each of the study participants was interviewed on a monthly basis by a trained interviewer and completed an open questionnaire investigating consumption of food and beverage items during the previous day from the time of waking up to going to bed at night (24-hour). Relative validity of FFQ and 24-hour diet recalls was determined by comparing nutrient intake obtained from these questionnaires with the results from biomarker measurements.[31]

Reported portion sizes of each food item intake were converted to grams based on raw-cooked coefficients (defined as the weight change after cooking the food item). Because the Iranian food composition table is incomplete and limited,[33] energy and nutrients content were assessed using Nutritionist software version IV (Nutritionist IV, Version 3.5.2) and the USDA food composition table (FCT)[34] which were adapted to Iranian foods. Although, for some local food items such as *Kashk*, vetch, wild plum, mint, sweet canned cherry and sour cherry which are not included in the USDA FCT, we used the Iranian version.

All participants were asked how much and how often, on average, they had eaten each food item during the previous year. Then, frequency of food intake was documented in times per day, week, month and year or never. In order to calculate the daily intake of each food item, the frequency of consumption was multiplied by the amount consumed according to the recorded portion sizes.

For this study, we categorized animal protein sources into six groups: red meat (beef, mutton/lamb, goat and game), processed meat (hamburgers, hot dogs, sausage, cold cuts and all types of meat products that has been supplied as ready-to-eat), chicken (poultry, chicken and hen), egg, total fish (all types of fish such as tuna, trout and processed fish such as conserved tuna, smoked and salted fish and all types of fish products that have been supplied as ready-to-eat), and organ meat (including sheep tongue and feet or *Kaleh Pacheh* ("head and leg", a meal consisting of sheep skull and tongue boiled together with knee joints), sheep intestines and stomach, as well as chicken or sheep liver and heart, lungs, testicles). Low or moderate fat dairy products consisted of low or moderate fat milk, low or moderate fat yogurt, high fat dairy products included high fat or whole milk, high fat yogurt, local cheese, and "*doogh*" (a drink made from salt, water and yoghurt). The nut group consisted of peanut, tree nut, and all nuts combined as well as legumes including soybeans, all types of beans, lentils, mug, split peas, peas and chickpeas. Plant based protein was calculated by subtracting the animal based proteins from total dietary protein.

Assessment of non-dietary factors

At the time of entry to the study, all subjects were asked to provide details of history of opium and tobacco use (having used at least weekly for a period of six months or more), alcohol use (having used at least weekly for a period of six months or more), as well as medical history of diagnosed disease (such as diabetes and cardio vascular diseases) and using medications. Lifetime pack-years of cigarette smoking were calculated by multiplying frequency of use per day by the duration of use in years. Socio-demographic characteristics (e.g., age, educational level, marital status, occupation, ethnicity, and residential area) were obtained from the general questionnaire. A composite score for wealth which presented socioeconomic status of population was calculated using multiple correspondence analysis based on ownership of appliances (i.e., personal car, motorbike, B/W TV, color TV, refrigerator, freezer, vacuum cleaner and washing machine in addition to each individual's house size, structure and ownership, and presence of a bathroom in residence and occupation).[35] A trained interviewer precisely determined and recorded each individual's height, weight, and waist and hip circumferences. Weight measurement was done using a standard scale with 100 g accuracy while minimally clothed without shoes. Height measurement was done while the subjects were in a standing position, without shoes and with the shoulders in a normal position, Waist circumference was measured using a standard tape at the level of the umbilicus. Then, we calculated body mass index (BMI) by dividing weight in kilograms by height in square meters (kg/m^2). Waist to hip ratio was calculated by dividing waist circumference by hip circumference.

Identification of pancreatic cancer cases

Since initiation, all GCS participants have been actively followed up based on phone calls every 12 months and a monthly review of the provincial death registration databases to check for their diagnosed medical conditions, including cancers, and vital status. If the subject was inaccessible, then next of kin, siblings,

friends or local health workers were contacted. The details of the study follow-up procedure and cancer have been discussed in depth previously.[29,36,37] In brief, when a person (or local health workers and in the case of death, next of kin) reported a diagnosis of pancreatic cancer on our follow-up contact, two professional physicians carried out a review of all medical records from hospitals and pathology laboratories in Golestan and other surrounding provinces in other to confirm the exact cause of death. Then, the research staff collected detailed data from the next of kin using a verbal autopsy questionnaire which was successfully validated. The information used for this study has been updated up to October 30, 2014. Finally, our trained medical team confirmed 54 incident pancreatic cancer cases (or deaths) diagnosed based on pathological and laboratory evidences, the local population-based cancer registry or the medical death documents.

Statistical Analysis

Baseline demographic and health characteristics were presented as mean ± SD and number (percent) for continuous and categorical variables, respectively.

All dietary items were reported in grams per day. Dietary intake of total protein, animal protein, plant based protein, red meat, organ meat, processed meat, poultry, egg, fish, dairy products, total nut, and legumes were categorized into tertiles based on the distributions of intakes in total cohort population.

Cox proportional hazard regression models were used to compute unadjusted and adjusted hazards ratios (HRs) and 95% confidence intervals (CIs) for the association between dietary intakes and risk of PC.

Follow-up time was calculated for each participant from the date they were recruited to the study until the date of death, failure to follow-up, or the end of follow-up date (30 Oct 2014), whichever came first.

HRs were reported by comparing the risk of progression for subjects in the highest tertiles relative to those in the lowest tertiles (that acted as the reference category).The median value of each tertile of total dietary intakes was used as a continuous variable to test for linear trends across categories.

For analyzing the association between consumption of different food items in dairy group and risk of PC, we used 3 category for each food item (< 5 g/d, 5–15 g/d, > 15 g/d) because of limited range of consumption.

The first regression models were only adjusted for age at recruitment (continues) and total energy intake (Kcal/d, continuous). The second multivariable models included adjustment for potential confounders, including history of diagnosed diabetes (yes or no), pack-years of cigarette smoking (0, .1–5, 5–10,10–20, > 20), years of education (0 (Illiterate), 1–5, 6–8, 9–12, University degree), alcohol consumption (ever use; defined as having used at least weekly for more than 6 months), opium use (ever use; defined as having used at least weekly for more than 6 months), body mass index (kg/m^2, continuous), age at recruitment (continuous), total energy intake (continuous), gender (male or female), metabolic equivalent task (MET)(continuous), wealth score (continuous) and residential area (urban or rural). Because adjusting the daily intake of total fiber, total fat, fruit and vegetable did not change the associations; therefore, these items were not included in the final Cox regression model.

All statistical tests were two-sided and p-values less than 0.05 were considered statistically significant. Analyses were performed using SPSS version 19 (SPSS Inc. 2011).

Results

At baseline, by increasing level of red and processed meat consumption and total protein intake, the percentage of men increased. In addition, the proportion of urban participants also increased with intake of red and processed meat and total protein than the subjects who resided in rural area (Table 1).

Subjects who consumed higher amounts of red and processed meat and total protein were more educated and had a higher score of wealth than those with lower consumption. Besides, this population was more likely to smoke and had higher intake of energy, total lipid, fruit and vegetables in comparison with the subjects with lower intake of red and processed meat and total protein. Similarly, alcohol consumption increased with red and processed meat and total protein intake. In addition, the subjects with higher consumption of total protein were more likely to be affected by diabetes and larger WHR than those with lower intake. On the contrary, the percentage of participants with a history of diabetes slightly lowered with increasing intake of red and processed meat. Furthermore, the individuals in the lowest tertile of red and processed meat consumption tended to be more physically active than those in the highest tertile. Opium use, age, and BMI were almost similar among or across the different tertiles of both total protein and red and processed meat consumption (Table 1).

Table 2 shows the associations between intake of different dietary protein sources and pancreatic cancer risk. During 383,630 person-years of follow-up, 54 cases of pancreatic cancer were ascertained. Person-years, adjusted incidence rates and the relative hazards of pancreatic cancer in 2 models of these dietary items' intake have been presented in Table 2.

There was a statistically significant decrease in risk of pancreatic cancer for the second versus lowest tertile of plant based protein intake in the first and multivariable models (HR = 0.27, 95% CI = 0.12–0.64, HR = 0.28, 95% CI = 0.12–0.65 respectively); however, this association was not significant anymore when comparing the highest tertile with the lowest, in the first and multivariable model (HR = 0.49, 95% CI = 0.19–1.24, HR = 0.52, 95% CI = 0.20–1.34, respectively) (Table 2).

However, there were no statistically significant associations between dietary intake of major protein sources (e.g. different types of meat, dairy products, egg, legumes and nuts) and risk of pancreatic cancer in age-energy adjusted analyses (Table 2). Further adjustment for other potential confounders including history of diabetes, pack-years of cigarette smoking, years of education, alcohol consumption, opium using, body mass index, age at recruitment, total energy intake, gender, MET, wealth score and residential area did not affect the association for the major dietary protein sources (data not shown). Moreover, the associations were not altered after additional control for other dietary variables such as dietary fiber, fruit, vegetable, and total fat intake. There was no statistically significant relationship between total and animal protein as well as intake of total grains and pancreatic cancer risk in both age-energy adjusted and the relative risk for one serving/day of total red meat intake.

Table 3 presents the association between different food items intake in dairy group and PC risk. However, consumption of these food items was also not associated with pancreatic cancer risk (data not shown).

Table 1. Baseline characteristics of study participants by tertiles of total protein and red and processed meat intake.

Characteristics	Total Protein intake(Tertile)			Red meat and processed meat consumption (Tertile)		
	1st	2nd	3rd	1st	2nd	3rd
Gender *n* (%)						
Female	11578 (71.4%)	9219 (56.8%)	7196 (44.3%)	10276 (62.8%)	9612 (59.3%)	8105 (50.3%)
Male	4646 (28.6%)	7005 (43.2%)	9032 (55.7%)	6086 (37.2%)	6587 (40.7%)	8009 (49.7%)
Ethnicity *n* (%)						
Turkmen	11962 (73.7%)	12168 (75.0%)	11919 (73.4%)	10064 (61.5%)	12792 (79.0%)	13193 (81.9%)
Other Ethnicities	4262 (26.3%)	4056 (25.0%)	4309 (26.6%)	6298 (38.5%)	3407 (21.0%)	2921 (18.1%)
Residence n (%)						
Rural	13412 (82.7%)	12964 (79.9%)	12406 (76.4%)	14186 (86.7%)	12892 (79.6%)	11704 (72.6%)
Urban	2812 (17.3%)	3260 (20.1%)	3822 (23.6%)	2176 (13.3%)	3307 (20.4%)	4410 (27.4%`)
Years of Education *n* (%)						
Illiterate	13054 (80.5%)	11141 (68.7%)	9903 (61.0%)	12836 (78.5%)	11284 (69.7%)	9978 (61.9%)
Below 5 years	2126 (13.1%)	2984 (18.4%)	3142 (19.4%)	2396 (14.6%)	2901 (17.9%)	2955 (18.3%)
6 to 8 years	409 (2.5%)	752 (4.6%)	1040 (6.4%)	503 (3.1%)	733 (4.5%)	965 (6.0%)
9 to 12 years	519 (3.2%)	1006 (6.2%)	1559 (9.6%)	509 (3.1%)	969 (6.0%)	1606 (10.0%)
University degree	116 (.7%)	341 (2.1%)	584 (3.6%)	118 (.7%)	312 (1.9%)	610 (3.8%)
History of Diabetes n (%)	971 (6.0%)	1003 (6.2%)	1400 (8.6%)	1451 (8.9%)	1002 (6.2%)	921 (5.7%)
Ever Smoker *n* (%)	2102 (13.0%)	2816 (17.4%)	3498 (21.6%)	2431 (14.9%)	2693 (16.6%)	3292 (20.4%)
Pack-years of smoking1 *n* (%)						
0	14122 (87.0%)	13408 (82.6%)	12730 (78.4%)	13931 (85.1%)	13506 (83.4%)	12822 (79.6%)
0.1–5	677 (4.2%)	888 (5.5%)	1137 (7.0%)	816 (5.0%)	885 (5.5%)	1001 (6.2%)
5.1–10	304 (1.9%)	425 (2.6%)	521 (3.2%)	328 (2.0%)	440 (2.7%)	482 (3.0%)
10.1–20	406 (2.5%)	618 (3.8%)	747 (4.6%)	517 (3.2%)	511 (3.2%)	743 (4.6%)
>20	715 (4.4%)	885 (5.5%)	1093 (6.7%)	770 (4.7%)	857 (5.3%)	1066 (6.6%)
Alcohol consumption *n* (%)	300 (1.8%)	526 (3.2%)	859 (5.3%)	319 (1.9%)	515 (3.2%)	851 (5.3%)
Opium using *n* (%)	2870 (17.7%)	2512 (15.5%)	2850 (17.6%)	2922 (17.9%)	2633 (16.3%)	2677 (16.6%)
Wealth score (quartiles) *n* (%)						
1st.	5673 (35.0%)	4077 (25.1%)	3548 (21.9%)	5508 (33.7%)	4162 (25.7%)	3628 (22.5%)
2nd.	4196 (25.9%)	3789 (23.4%)	3388 (20.9%)	4543 (27.8%)	3734 (23.1%)	3096 (19.2%)
3rd.	3563 (22.0%)	4124 (25.4%)	4153 (25.6%)	3817 (23.3%)	4134 (25.5%)	3889 (24.1%)
4th.	2792 (17.2%)	4234 (26.1%)	5139 (31.7%)	2494 (15.2%)	4169 (25.7%)	5501 (34.1%)
MET (hours/d, Mean ± SD)	28.58±10.81	29.87±12.87	29.95±13.50	30.32±13.33	29.23±12.05	28.83±11.90
Age (years; Mean ± SD)	52.65±9.061	51.56±8.73	51.88±8.85	53.08±9.12	51.84±8.81	51.15±8.62
Body mass index (kg/m^2; Mean ± SD)	26.10±5.52	26.79±5.38	27.15±5.20	26.15±5.40	26.80±5.41	27.10±5.31
WHR (m, Mean ± SD)	0.94±.08	0.95±.08	0.96±.07	0.95±.08	0.95±.08	0.95±.08
Dietary Intakes (g/d, Mean ± SD)						
Energy	1644.35±374.31	2155.60±321.79	2689.72±522.69	1945.53±557.29	2152.09±544.50	2395.66±595.85
Total Protein	51.59±10.05	73.36±5.20	103.38±24.40	69.90±26.92	74.52±23.98	84.03±25.91
Total fat	58.94±17.98	74.79±17.14	93.26±23.14	66.35±22.50	75.22±21.61	85.57±24.17
Red and processed meat	10.13±10.08	14.09±12.69	20.41±25.82	3.09±1.95	10.71±2.66	31.03±23.66
Fruit	103.64±87.76	145.27±110.68	204.59±160.60	104.57±92.11	147.49±120.94	202.20±151.99
Vegetables	152.32±70.00	182.80±76.92	217.97±97.99	163.87±80.83	182.55±81.34	207.02±92.22
[1]Among current and past smokers						

Table 2. Baseline intakes of total and different sources of proteins and the risk of pancreatic cancer.

Variables	Tertiles of Intake			*P for trend*
	Tertile 1	Tertile 2	Tertile 3	
Total Protein				
Median (g/d)	53.97	73.25	96.31	
No. of cases/cohort	26/16198	13/16211	15/16213	
Person-years	126305	127801	129524	
Age-Energy Adjusted Model HR[a](CI)	1.00	0.55 (0.26–1.16)	0.63 (0.25–1.59)	0.32
Multivariable Adjusted Model HR[b](CI)	1.00	0.64 (.30–1.38)	0.86 (.33–2.24)	0.76
Animal protein				
Median (g/d)	17.06	28.83	45.45	
No. of cases/cohort	18/16205	19/16205	17/16211	
Person-years	127795	127667	128160	
Age-Energy Adjusted Model HRa (CI)	1.00	1.15 (0.59–2.23)	1.03 (0.51–2.11)	0.95
Multivariable Adjusted Model HRb (CI)	1.00	1.31 (0.67–2.58)	1.31 (0.62–2.77)	0.49
Plant based Proteins				
Median (g/d)	29.95	43.88	56.27	
No. of cases/cohort	31/16193	8/16215	15/16213	
Person-years	126179	127196	130247	
Age-Energy Adjusted Model HRa(CI)	1.00	0.27 (.12-.64)	0.49 (.19-1.24)	0.05
Multivariable Adjusted Model HRb (CI)	1.00	0.28 (0.12-.65)	0.52 (0.20-1.34)	0.07
Total red meat				
Median (g/d)	1.73	6.86	19.08	
No. of cases/cohort	22/16168	19/16602	13/15848	
Person-years	123537	131221	128841	
Age-Energy Adjusted Model HRa(CI)	1.00	0.89 (0.48–1.66)	0.68 (0.33–1.38)	0.28
Multivariable Adjusted Model HRb (CI)	1.00	0.76 (0.40–1.44)	0.54 (0.25–1.16)	0.13
Processed meat				
Median (g/d)	.00	1.34	7.63	
No. of cases/cohort	24/20402	13/12025	17/16191	
Person-years	157176	97128	129292	
Age-Energy Adjusted Model HRa (CI)	1.00	1.16 (0.59-2.31)	1.28 (0.67-2.45)	0.47
Multivariable Adjusted Model HRb (CI)	1.00	1.07 (0.53–2.12)	1.16 (0.60–2.22)	0.66
Total Poultry and Chicken				
Median (g/d)	16.48	48.09	96.18	
No. of cases/cohort	16/16212	19/16218	19/16189	
Person-years	131492	127382	124732	
Age-Energy Adjusted Model HRa (CI)	1.00	1.23 (0.63–2.41)	1.15 (0.58–2.27)	0.72
Multivariable Adjusted Model HRb (CI)	1.00	1.41 (0.72–2.78)	1.53 (0.76–3.08)	0.25
Total Fish				
Median (g/d)	0.00	3.30	14.82	
No. of cases/cohort	18/15745	23/17757	13/15115	
Person-years	124742	139784	119061	
Age-Energy Adjusted Model HRa (CI)	1.00	1.33 (0.71–2.48)	0.94 (0.45–1.95)	0.66
Multivariable Adjusted Model HRb (CI)	1.00	1.39 (0.73–2.62)	1.02 (0.47–2.21)	0.81
Organ meat				
Median (g/d)	.20	2.48	9.21	
No. of cases/cohort	23/16319	15/16188	16/16111	
Person-years	128804	128170	126623	
Age-Energy Adjusted Model HR[a] (CI)	1.00	0.69 (0.36–1.32)	0.76 (0.40–1.45)	0.53
Multivariable Adjusted Model HR[b] (CI)	1.00	0.68 (0.35–1.31)	0.75 (0.39–1.43)	0.51
High Fat Dairy products				
Median (g/d)	17.86	75.00	200.00	
No. of cases/cohort	18/16242	16/16179	20/16198	
Person-years	127620	126544	129441	
Age-Energy Adjusted Model HR[a] (CI)	1.00	0.98 (.50–1.94)	1.27 (.65–2.48)	0.42
Multivariable Adjusted Model HR[b] (CI)	1.00	0.77 (0.39–1.54)	0.77 (0.39–1.51)	0.42

Low and Moderate Fat Dairy products				
Median (g/d)	18.18	68.93	140.00	
No. of cases/cohort	22/16204	14/16206	18/16207	
Person-years	125812	125163	132612	
Age-Energy Adjusted Model HR[a] (CI)	1.00	0.68 (.35–1.34)	0.86 (.45–1.64)	0.70
Multivariable Adjusted Model HR[b] (CI)	1.00	0.67 (0.34–1.33)	0.87 (0.42–1.78)	0.73
Egg				
Median (g/d)	0	9	21	
No. of cases/cohort	23/17082	14/17185	17/14349	
Person-years	131857	135724	116004	
Age-Energy Adjusted Model HR[a](CI)	1.00	0.77 (.39–1.51)	1.26 (0.65–2.45)	0.50
Multivariable Adjusted Model HR[b] (CI)	1.00	0.73 (0.37–1.44)	1.18 (0.60–2.30)	0.63
Nuts				
Median (g/d)	.00	2.12	11.78	
No. of cases/cohort	24/15827	13/16654	17/16133	
Person-years	119107	132650	131809	
Age-Energy Adjusted Model HR[a] (CI)	1.00	0.75 (.37–1.51)	1.09 (.56–2.11)	0.57
Multivariable Adjusted Model HR[b] (CI)	1.00	0.78 (.38–1.58)	1.14 (0.58–2.25)	0.49
Total Legumes				
Median (g/d)	6.21	14.54	27.14	
No. of cases/cohort	22/16426	20/16091	12/16104	
Person-years	129359	125836	128433	
Age-Energy Adjusted Model HR[a] (CI)	1.00	0.99 (.54–1.82)	0.59 (.29–1.21)	0.15
Multivariable Adjusted Model HR[b] (CI)	1.00	1.04 (.56–1.93)	0.65 (.31–1.37)	0.26
Total grains				
Median (g/d)	289.41	431.58	533.76	
No. of cases/cohort	28/16196	12/16212	14/16214	
Person-years	125971	127577	130081	
Age-Energy Adjusted Model HR[a] (CI)	1.00	0.51 (0.24–1.07)	0.64 (0.27–1.51)	0.19
Multivariable Adjusted Model HR[b] (CI)	1.00	0.52 (0.25–1.11)	0.65 (0.27–1.55)	0.21

[a] Cox regression adjusted for age at recruitment (continues), total energy intake (continues).
[b] Cox regression adjusted for history of diabetes (yes or no), pack-years of cigarette smoking (0, .1–5, 5–10,10–20, >20), years of education (0(Illiterate), 1–5, 6–8, 9–12, University degree), alcohol consumption (ever use; defined as having used at least weekly for more than 6 months), opium using (ever use; defined as having used at least weekly for more than 6 months), body mass index (kg/m2, continues), age at recruitment (continues), total energy intake (continues), gender (male or female), MET (continues), wealth score (continues) and residential area (urban or rural).

Discussion

In this large prospective cohort, we did not observe clear and consistent evidence for an association between main dietary protein intakes and the risk of PC. There was only a statistically significant inverse association between risk of PC for the second versus lowest tertile of plant based protein intake; however, this association was not significant anymore when comparing the highest tertile with the lowest.

Case-control studies have mainly observed positive associations between red meat consumption and PC risk[12–14]; however, these studies may have suffered from information bias, either by relying on proxy interviews, or by disease-related alterations in their dietary habits. Most of the prospective cohort studies, which have overcome these limitations, did not find a statistically significant association between dietary main animal sources of protein intake and PC risk[20,23,24,38]; only Larsson *et al.* have shown a positive association of red meat consumption and an inverse association of poultry consumption, with risk of PC in a cohort of Swedish women[18]; however, they[25] showed in a meta-analysis that red meat consumption was not associated with risk of PC overall, but was positively associated with risk in men. Another meta-analysis has also shown no association between consumption of red meat, fish, poultry, and eggs in cohort studies.[26] Our results support the previous reports of no association between animal protein intake and PC risk. It is possible that the true relationship between intake of meat and pancreatic cancer might be confounded by the cooking methods. As it was concluded in our previously published case-control study, barbecuing red meat and frying vegetables and fish were related to increased risk of PC.[39] Barbecuing red meat may produce high amounts of carcinogens such as heterocyclic amines (HCAs) and polycyclic aromatic hydrocarbons (PAHs).[40–45] Thus, it is important for future studies to investigate the different cooking methods and its association with PC risk.

Furthermore, other factors including the participant's lifestyles and their whole dietary pattern, as well as the use of hormones in animal husbandry[46] may affect the relationship between dietary protein intake and PC risk. However, considering some of these lifestyle-related risk factors such as socio-economic status, smok-

Table 3. Baseline intakes of different sources of dairy intake and the risk of pancreatic cancer.

Food item Intake(g/d)	No. of cases/cohort	Person-years	Age-Energy Adjusted Model HR[a] (CI)	Multivariable Adjusted Model HR[b] (CI)
Low and Moderate fat milk				
<5 g/d	47/43160	337400	1.00	1.00
5-15 g/d	2/759	6292	2.48 (0.60–10.24)	2.72 (0.59–12.44)
>15 g/d	5/4694	39867	.88 (0.35-2.21)	1.04 (0.34–3.14)
P for trend		0.79		0.96
High Fat Milk				
<5 g/d	15/13746	110203	1.00	1.00
5-15 g/d	4/5797	44791	0.68 (0.22–2.07)	0.66 (0.21–2.03)
>15 g/d	35/29074	228595	1.17 (0.63–2.17)	1.17 (0.61–2.24)
P for trend		.41		0.40
Low and Moderate fat Cheese				
<5 g/d	23/16435	129359	1.00	1.00
5-15 g/d	17/21467	168540	.64 (.34–1.22)	.66 (.34–1.25)
>15.1 g/d	14/10714	85680	1.16 (.58–2.30)	1.16 (.57–2.37)
P for trend		.68		.66
Low and Moderate fat Yoghurt				
<5 g/d	11/7473	59176	1.00	1.00
5-15 g/d	2/2409	18493	0.53 (0.11–2.40)	0.52 (0.11–2.38)
>15 g/d	41/38733	305902	0.74 (.38–1.46)	0.73 (0.37–1.45)
P for trend		0.56		0.53
High Fat "Dough"				
<5 g/d	13/12635	101985	1.00	1.00
5-15 g/d	14/6408	49868	2.33 (1.09–4.98)	2.44 (1.14–5.20)
>15 g/d	27/29575	231744	1.06 (0.54–2.07)	1.05 (0.53–2.08)
P for trend		0.48		0.46
High Fat Yoghurt				
<5 g/d	41/38348	299439	1.00	1.00
5-15 g/d	4/3993	32770	0.96 (0.34–2.69)	0.89 (0.31–2.52)
>15 g/d	9/6277	51394	1.40 (0.67–2.90)	1.36 (0.65–2.84)
P for trend		0.35		0.40

[a] Cox regression adjusted for age at recruitment (continues), total energy intake (continues).
[b] Cox regression adjusted for history of diabetes (yes or no), pack-years of cigarette smoking (0, .1–5, 5–10,10–20, >20), years of education (0(Illiterate), 1–5, 6–8, 9–12, University degree), alcohol consumption (ever use; defined as having used at least weekly for more than 6 months), opium using (ever use; defined as having used at least weekly for more than 6 months), body mass index (kg/m2, continues), age at recruitment (continues), total energy intake (continues), gender (male or female), MET (continues), wealth score (continues) and residential area (urban or rural).

ing, alcohol consumption, opium use, BMI, dietary factors, and physical activity level as confounding variables, did not effectively alter the results of the regression models.

In addition, as it has been shown, the median intake of different meat items was low in this population, while consumption of grains was high so grains could provide a source of protein for this population. Thus, we included the grain intakes as a dietary protein source in our analysis; however, we did not observe any statistically significant relationship between grains intake and risk of PC. Previous studies have shown that intake of white bread and refined grains are positively associated with PC risk.[47–49] This inconsistency between our findings and these three studies may be related to the fact that the median intake of grains in the GCS participants is lower than the mentioned studies.

We found a statistically significant inverse association between risk of PC for the second versus lowest tertile of plant based protein intake; however, there was no significant association between PC risk and intake of nuts and legumes which is in contrast with previous study by Bao *et al.* who[4] reported an inverse association between frequency of nut consumption and PC risk. It seems that this weak inverse association between plant based protein intake and PC risk, in this study, might be due to the protein of vegetables, and it should be explained by their antioxidant contents.

The present study had several strengths, including its prospec-

tive design, large sample size, high participation and follow-up rate. In addition, one of the most important strengths of this analysis is that it is the first study to assess the association of main dietary protein intakes and PC risk in a Middle Eastern country with its special dietary habits. Studies in developing countries can provide unique opportunities to test for associations between diet and disease[50] within the context of different lifestyle patterns. People in developing countries tend to have different socio-economic backgrounds of people than those in developed Western world, and these differences can help establish the independence of a putative association. These differences highlight the importance of examining associations in populations with different lifestyles.

Our study also had several limitations. The most important limitation of this study is that we did not assess the method of cooking for different food items, which could affect the nutrient content of foods and production of toxic substances. Another shortcoming of this study is that the duration of follow-up in GCS is still relatively short. Therefore, we cannot exclude that associations may be found after more years of follow-up, whereas exposure misclassification may increase with longer follow-up. It is also possible that in cases with a relatively short duration of follow-up, disease-related changes in diet have occurred, resulting in inaccurate dietary assessment. However, restriction of our analyses to individuals with a follow-up duration of more than 2 years did not materially change the results. There remains the possibility of residual confounding and other non-causal explanations. Also, this study was conducted in an older population in a high-risk region for cancer, so the results cannot be necessarily extrapolated to other populations. However, we do note that previous studies in developed, Western countries, have found similar results. Another possible limitation of this study is that the height measurement might not be accurate due to the possible presence of some degree of osteoporosis in old people. And finally, since the dietary intakes were self-reported, some measurement error was inevitable.

In conclusion, we did not observe any clear and consistent evidence for an association between main dietary protein intakes and the risk of pancreatic cancer. Examining the method of cooking is recommended in future studies.

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