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Pancreatic cancer expresses adiponectin receptors and is associated with hypoleptinemia and hyperadiponectinemia: a case-control study

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Abstract

Obesity and insulin resistance have been implicated in the etiology of pancreatic cancer (PC). Whether adiponectin and/or leptin, two adipocyte-secreted hormones important in metabolic regulation, are associated with PC pathogenesis and whether adiponectin receptors are expressed in PC remains unknown. In a hospital-based case-control study, we studied 81 cases with incident, histologically confirmed PC and 81 controls matched on gender and age between 2000 and 2007 to investigate the role of adiponectin and leptin adjusting for risk factors linked to PC. In a separate study, we also studied for the first time whether adiponectin receptors 1 and 2 are expressed in PC by studying 16 PC tumor tissue samples which were analyzed using immunohistochemistry. When subjects were divided into control-defined quartiles of adiponectin and leptin, lower leptin but higher adiponectin levels were associated with PC ($p=0.001$ and $p=0.05$ respectively) before and after controlling for age, gender, BMI, smoking status, alcohol consumption, history of diabetes, and family history of pancreatic cancer. Of the PC tumor tissue samples analyzed, 87.5% had positive or strong positive expression of AdipoR1 and 93.7% had positive or strong positive expression of AdipoR2. Further prospective studies are needed to determine whether the elevated adiponectin and low leptin levels reported in this study reflect compensatory changes during PC progression and thus can be used as markers for PC or whether they are causally implicated in PC.

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Keywords

leptin; adiponectin; adipokine; pancreatic cancer; obesity

Introduction

Pancreatic cancer (PC) is the fourth leading cause of cancer death in Western countries (1). Its incidence has been rising in the past few decades; it has a very dismal prognosis, and a five-year survival rate less than 5% (1–3). Survival rates have not improved during the past 25 years; localized resectable tumor has only a 17% survival rate (1,4).

Pancreatic adenocarcinoma is the most common exocrine malignancy, accounting for 80% of all pancreatic cancers (1–3). PC rarely occurs in individuals less than 50 years and its incidence peaks in the senenth and eighth decades of life (1,4). Little is known about the etiology of most cases of PC. Several demographic features such as age, gender, and race have been implicated in the etiology of PC but cigarette smoking represents the only established environmental risk factor (5,6). A history of diabetes mellitus (DM) and obesity, particularly in men, are associated with increased risk of PC (6–10). High insulin concentrations and insulin resistance have also been shown to be predictive of PC risk among male smokers (11). At this time, there is not sufficient evidence to support an association between alcohol or coffee consumption and PC (4,6), but high fruit and vegetable consumption as well as physical activity may reduce PC risk (4,10). Rarely, hereditary factors such as a family history of PC, hereditary chronic pancreatitis and certain familial cancer syndromes may also result in a substantially augmented risk of PC (12–13).

Adipose tissue is now broadly recognized as an active endocrine organ secreting several bioactive adipokines, which regulate physiological and pathological processes, such as appetite, insulin sensitivity and resistance, inflammation, immunity, hematopoiesis and angiogenesis (14–15). The two most studied adipokines are leptin and adiponectin. Leptin regulates multiple physiologic functions in humans, mainly in states of energy deficiency, since currently available evidence indicates that leptin has only a permissive role in humans (14, 16). Low leptin levels are significantly and causally associated with insulin resistance (16). Adiponectin is a pleiotropic, insulin-sensitizing, anti-inflammatory and anti-atherogenic adipokine (17). Adiponectin has been found to have a protective role in several types of malignancies in vivo, notably those related to obesity in six case-control studies and one prospective study (18–24), including childhood myeloblastic leukemia in a case-control study (25) and myelodysplastic syndrome in a case-control study (26). On the contrary, a recent case-control study reported that serum adiponectin was significantly elevated in PC, a malignancy which is generally associated with obesity, and suggested that adiponectin could be used as a potential tumor marker in PC (27,28). In contrast, another, prospective study, reported that prospectively collected prediagnostic serum adiponectin levels are inversely associated with PC (29). To our knowledge, no prior study has jointly evaluated leptin and adiponectin in relation to pancreatic cancer and no previous study has examined whether adiponectin receptors are expressed in PC tumor tissue.

Since no previous study has jointly evaluated adiponectin and leptin in relation to PC pathogenesis, we attempted to explore whether these adipose tissue hormones are associated with PC, taking into account other known risk factors. In this case-control study, we investigated the role of serum leptin and adiponectin levels in the pathogenesis of PC after adjusting for body mass index (BMI) at one year prior to interview, history of diabetes mellitus, family history of gastrointestinal cancer or PC, alcohol consumption and smoking status, and evaluated their correlation with tumor markers and prognostic factors. We hypothesized that

since PC is related to obesity and insulin resistance, levels of both adipokines would be decreased in PC. We also hypothesized that adiponectin receptors would be expressed in PC tumor tissue. Future cohort studies should be conducted in order to evaluate prospectively the association of serum adipokines with PC risk.

Materials and methods

A. Hospital based case-control study

Patients and controls—Cases and controls were recruited from patients hospitalized at the Veterans' Administration General Hospital of Athens (NIMTS). This hospital is the only Veteran's Hospital in Athens Metropolitan area and all of Southern Greece. The study recruited 81 cases and 81 controls under 85 years of age from the same study base. All were of Greek nationality and were permanent residents of Greece. Medical records were reviewed and interviews were carried out to obtain information on demographic characteristics, medical history, presence of diabetes mellitus, average consumption of alcoholic beverages (in glasses per day), tobacco smoking, present weight and height, as well as weight and height one year prior to enrollment in the study and interview. Family history of gastrointestinal (GI) cancer including pancreatic cancer was collected for first-degree relatives (parents and siblings) and for second-degree relatives (grandparents, uncles and aunts).

Eligible cases included newly diagnosed patients who were operated on and histologically confirmed to have pancreatic adenocarcinoma, under age 85, consecutively admitted to the Internal Medicine Department of the Veterans' Hospital between 20 May 2000 and 4 November 2007. A total of 89 cases were identified and of those 81 cases (49 males and 32 females), aged 47–83 years (median age, 71) consented to participate and were interviewed. The main reasons for nonparticipation were inability of the patient to communicate/participate and/or refusal on the part of the subject or his/her relatives. Nevertheless, responders and non-responders did not differ on demographic variables, notably age, gender and time of diagnosis.

Controls were patients, under age 85, admitted for non-neoplastic and non-infectious conditions to the Orthopedic, Surgical and Ophthalmologic Department of the same hospital and matched to cases on age (± 5 years), gender and year/month of diagnosis (± 1 month). No control subjects developed pancreatic cancer. The main causes of admission to the hospital in the control group were: scheduled senile cataract operation (22.2%), scheduled hernia operation (12.3%), scheduled hip (21%) or knee joint replacement (9.9 %) due to idiopathic (primary) osteoarthritis, injuries, in particular fractures not secondary to a disease (27.2%) and sciatica (7.4%). For every eligible case, an attempt was made to randomly identify a control admitted to the Veterans' Administration General Hospital as closely as possible in time to the admission of the corresponding case (± 1 month). A total of 92 potential controls were identified and of those 81 consented to participate and were interviewed. Among the latter, 49 were males and 32 females, aged 47–85 years (median age, 72).

All cases and controls who participated in our investigation were fully informed of the aim of the study and gave written consent for their participation and their agreement that, provided that their anonymity is maintained, the results of this study may be presented or published, solely in the interest of science.

Diagnostic procedure, specimen collection and laboratory analysis—Pancreatic adenocarcinoma was histologically confirmed by pathologic examination of the resected specimen or, if unresected, by intraoperative biopsies. Staging was based upon laparotomy and imaging methods such as contrast-enhanced CT. Pancreatic cancer patients were also classified in four prognostic stages according to the clinical staging system of the Union International

Cancer Classification (28). Of the 81 patients, 8 patients had stage II (9.9%), 32 patients had stage III (39.5%) and 41 patients had stage IV (50.6%) tumors.

All blood specimens were collected prior to the initiation of chemotherapy and/or radiotherapy for the cases and prior to any therapeutic approach, including surgery, for the control group. Peripheral blood samples were centrifuged in the laboratory. Serum was separated and stored at -80°C . For cases, preoperative serum values of carcinoembryonic antigen (CEA), CA 19-9 and CA 72-4 used in the diagnostic work-up, were abstracted from medical records. For controls, serum CEA, CA 19-9 and CA 72-4 were measured by electrochemiluminescence immunoassay intended for use on Elecsys 2010 analyzer (Roche Diagnostics, Indianapolis, USA). The sensitivity was 0.2 ng/mL for CEA, 0.6 U/mL for CA 19-9 and 0.2 U/mL for CA 72-4. Normal ranges in our laboratory, defined as the mean $\pm$ 2 S.D. values for healthy controls, were 0.2–3.4 ng/mL for CEA in non-smokers, 0.2–4.3 ng/mL for CEA in smokers, 0.6–27 U/mL for CA 19-9 and 0.2–8.2 U/mL for CA 72-4. All coded samples were shipped in one batch to the Beth Israel Deaconess Medical Center, in Boston, USA. Serum adiponectin was determined by radioimmunoassay (LINCO Research, St. Charles, MO) with a sensitivity of 1 ng/mL, an intra-assay coefficient of variation of 1.8–6.2%, an inter-assay coefficient of variation of 6.9–9.3% and a recovery rate of 99–103% for adiponectin. Serum leptin levels were measured using radioimmunoassay (LINCO Research, St. Charles, MO). For leptin, the sensitivity of the assay was 0.5 ng/mL, with an intra-assay coefficient of variation of 3.4–8.3% and an inter-assay coefficient of variation of 7.0–9.3%.

B. Immunohistochemistry study

Immunohistochemistry analysis—Sixteen formalin-fixed paraffin-embedded pancreatic adenocarcinoma and ductal adenocarcinoma tissue specimens were available for analysis from American male and female patients with pancreatic carcinoma. All specimens were purchased in the form of tissue array slides mounted to standardized silanized slides (Imgenex, San Diego, CA, USA).

The 5 μm paraffin tissue sections were deparaffinized, rehydrated, microwaved for 25 min. in 10 mmol/l citrate buffer, and incubated for 30 min. in methanol containing 0.5% H_2O_2 . The slides were incubated in 16% normal goat serum for 1 hour at room temperature, then incubated for 1 hour with the primary antibodies at room temperature. The primary antibodies used were the rabbit anti-human AdipoR1 (raised against amino acid residues 357–375) antiserum and the rabbit anti-human AdipoR2 (raised against amino acid residues 374–386) antiserum (both from Phoenix Pharmaceuticals Inc., Belmont, CA, USA) used at 1:500 and 1:200 dilution respectively. The secondary antibody was a biotinylated anti-rabbit antibody (1:400 dilution) and was applied for 30 min. at room temperature, followed by the Vectastain Elite ABC Reagent (Vector Laboratories, Burlingame, CA, USA) for 30 min. The POD reaction was developed with diaminobenzidine, and the slides were counterstained with hematoxylin. Intensity and distribution of positive staining was evaluated on a scale of 0 to +++ by an expert pathologist.

Statistical analysis—Descriptive characteristics of PC case and control subjects are presented as proportions or as mean values $\pm$ standard deviation (SD). Comparisons between cases and controls were conducted using chi-square tests for categorical variables and t-tests for continuous variables. One-way ANOVA with Bonferroni correction was conducted to compare cases among different prognostic subgroups. Nonparametric Spearman correlation coefficients were calculated to examine the associations of hormones and anthropometric characteristics among the controls. Subjects were divided into control-defined quartiles of leptin and adiponectin for analysis and multivariate unconditional logistic regression was used to compare cases and controls with respect to hormone levels. Crude OR estimates were

adjusted for matching factors and adjusted OR estimates were adjusted for matching factors (age, gender and date of diagnosis), as well as anthropometric, lifestyle, and medical history variables such as BMI at one year prior to interview, history of diabetes mellitus, family history of gastrointestinal cancer, alcohol consumption, smoking status, and adiponectin/leptin. In order to evaluate statistical interaction between leptin and adiponectin, we entered into the statistical model the main effect terms and a term representing the cross-product, the coefficient for which was tested by the Wald test. Adiponectin receptor expression between adenocarcinoma and ductal adenocarcinoma pancreatic tissue was compared using Fisher's exact test. Statistical analysis of the data was performed with SAS 9.1 for Windows XP (SAS Institute, Cary, N.C.)

Results

Cases and controls were matched by gender, age (within 5 years) and date of diagnosis (within 1 month). Cases had significantly higher initial BMIs (one year prior to enrolment in the study) than controls, but there were no significant differences in BMI at the time of the interview. Similarly, initial weight was significantly higher among cases, but there were no significant differences in weight at the time of the interview. Subjects with PC also had significantly higher adiponectin and lower leptin concentrations than controls. Controls exhibited significantly lower concentrations of CEA as well as tumor markers CA 19-9 and CA 72-4. Cases were more likely to have a history of diabetes, a family history of pancreatic or GI cancer, and more likely to smoke one or more packs of cigarettes per day (Table 1). Spearman correlation coefficients were calculated among cases and controls. Among cases, the tumor marker CA 19-9 was significantly positively correlated with CA 72-4 ($r=0.29$, $P=0.02$) and with CEA ($r=0.35$, $P=0.001$).

Table 2 summarizes the odds ratios for pancreatic cancer by control-defined quartiles of adiponectin and leptin concentrations, with and without covariate adjustment. Subjects in the highest quartile of adiponectin concentration present a significantly higher odds for pancreatic cancer before (OR=2.66, 95% CI 1.11-6.34, $p=0.03$) and after adjustment for BMI at one year prior to interview, history of diabetes mellitus, family history of pancreatic or GI cancer, alcohol consumption, smoking status, and leptin (OR=2.81, 95% CI 1.04-7.59, $p=0.04$). In contrast, subjects in the highest quartile of leptin concentration have a lower odds for pancreatic cancer, before (OR=0.12, 95% CI 0.04-0.36, $p=0.0002$) and after adjustment for covariates (OR=0.16, 95% CI 0.05-0.54, $p=0.003$). Similar results were obtained when subjects were divided into control-defined tertiles. For leptin, there was a continuous decrease in odds of PC as leptin concentrations increased. For adiponectin, however, there appears to be a threshold effect resulting in a large increase in the odds of PC in the 4th quartile. No significant interaction was found between adiponectin and leptin, with respect to pancreatic cancer status.

Comparisons of mean adiponectin and leptin levels among pancreatic cancer patients in different prognostic stages revealed no significant differences, based on the overall ANOVA test (Table 3). Also, pair wise comparisons did not exhibit any significant differences between subgroups.

Table 4 presents data on descriptive characteristics and pancreatic tissue adiponectin receptor expression for 16 patients with pancreatic cancer. Tissue samples analyzed were from ductal adenocarcinoma (62.5%) and adenocarcinomas which were not further classified (37.5%). Most were stage II (56.3%) or III (37.5%), and moderately differentiated (81.3%). The majority of the pancreatic tumor tissue had positive or strong positive expression of AdipoR1 (87.5%) and AdipoR2 (93.7%). All pancreatic tumor tissue analyzed showed at least marginal expression of AdipoR2, and all but 2 samples showed at least marginal expression of AdipoR1.

Discussion

The results of this case-control study demonstrate that higher serum adiponectin levels and low leptin levels are associated with higher odds of pancreatic cancer before and after controlling for age, gender, date of diagnosis, BMI at one year prior to interview, history of diabetes mellitus and family history of gastrointestinal or pancreatic cancer as well as alcohol consumption and smoking status. This finding regarding adiponectin is in accordance with a previous case-control study showing that adiponectin was significantly higher in 72 patients with PC as compared to control subjects (27) and extends these by demonstrating that this association is independent of potential confounders. This case-control study also confirms the previously reported positive association of tobacco smoking, history of diabetes mellitus and family history of pancreatic cancer with PC (4–7,12), as well as the cross-correlation between prognostic factors among cases, all of which supports the validity of the study. However, our finding regarding hyperadiponectinemia in pancreatic cancer cases is in disagreement with a very recent cohort study of male Finnish smokers who provided blood samples for more than five years prior to pancreatic cancer diagnosis (29). In this study, lower prediagnostic adiponectin levels were associated with pancreatic cancer risk adjusting for smoking, blood pressure and C-peptide levels. We cannot underestimate the strength of the prospective design of this study and the large number of incident pancreatic cancer patients but the two study populations are not directly comparable; this study consists of Finnish male smokers, whereas our case-control study comprises Greek men and women, smokers and non-smokers. Another interesting finding is that no association was observed for pancreatic cancer risk and BMI in the Finnish population (29–30). One could speculate whether pancreatic cancer pathogenesis is different in these substantially different populations or whether, similar to other malignancies, low adiponectin levels at baseline are associated with obesity and predict PC but adiponectin levels increase rapidly either to compensate for insulin resistance and/or in response to loss of weight in more advanced stages of their disease.

Taking into account the totality of available evidence, the finding that pancreatic cancer is associated with elevated adiponectin levels was unanticipated. Hypoadiponectinemia has previously been reported to be associated with obesity, type II diabetes mellitus, and cardiovascular disease (31) as well as with several malignancies related to obesity in several studies with serum collected at diagnosis and in one study with prospectively collected serum (14,17–26). Several epidemiologic studies have shown inverse associations between adiponectin levels and risk for breast (18), endometrial (19), prostate (20), gastric (21), renal (22), colorectal (23) cancers as well as childhood AML (25), myelodysplastic syndrome (26) and melanoma (24). The positive association between PC and adiponectin is also surprising since PC may be related to obesity and insulin resistance. Most studies have reported a positive association between PC and BMI particularly in men and nonsmokers (9–10,32) while other studies have shown a weak association with obesity or no association (8). High insulin concentrations and insulin resistance have also been shown to be predictive of PC risk among male smokers (11). Moreover, in a recent prospective study, prediagnostic nonfasting serum C-peptide was positively associated with pancreatic cancer risk in men and women (33). In our study, initial BMI one-year prior to PC presentation was associated with increased risk of PC in accordance with other studies (9–10) while BMI at interview, ie. after significant weight loss due to cancer, was not significantly different for cases than for controls. Potential mechanisms to link obesity and PC include insulin resistance, which is considered as a risk factor for PC (6–10) as well as several endocrine factors that could play an important underlying role in this regard, including adipokines as well as the IGF-I system which may have mitogenic and antiapoptotic properties (34).

Adiponectin, an adipocyte-secretory hormone, has anti-atherogenic, vasculo-protective and anti-inflammatory properties, and has been demonstrated to improve insulin sensitivity (14–

15,17). Although low adiponectin levels have been linked to obesity-related diseases, elevated adiponectin levels have been associated with mortality in studies of selected groups of patients with various diseases at baseline (35–37). Increased adiponectin concentrations were strongly related to all-cause and cardiovascular mortality in patients with coronary artery disease at baseline (35), and all-cause mortality in patients with chronic heart failure (36) and chronic kidney disease (37). These findings may be attributable to the role of adiponectin as a marker for wasting (36) and/or as a factor which could compensate for increasing insulin resistance in these circumstances. Similarly, adiponectin levels may have been elevated among cases in this study to compensate for insulin resistance and/or in response to the disease-induced weight loss possibly through altering the size of adipocytes. This association could also be interpreted as a compensatory response to the inflammation and/or weight loss due to cancer cachexia (38), a complex metabolic state characterized by loss of adipose and muscle tissue (38). This is supported by previous findings showing that anorexia nervosa as well as prolonged voluntary weight loss are associated with high adiponectin levels (39). The association with adiponectin persisted, however, even after adjusting for BMI and leptin levels. An alternative explanation for the association between elevated adiponectin levels and PC could be adiponectin resistance produced by a down regulation of adiponectin receptors or signaling pathways downstream of the receptors leading to subsequent counter-regulatory increased adiponectin secretion. We report, however, that adiponectin receptors are present in PC and that both adiponectin receptors are strongly expressed in the vast majority of studied subjects. Finally, we cannot exclude that adiponectin receptors present in PC along with hyperadiponectinemia may represent an adaptive process to cancer.

We have also found that low leptin levels are associated with increased odds of PC, independently of other factors, as seen in two prior studies examining serum leptin in PC with far fewer patients (7 and 64 patients with PC respectively) (40–41). Leptin, a 16-kd protein encoded by the *obese* gene, is produced mainly by white adipocytes and is positively correlated with body fat mass (16). It has been recently demonstrated that leptin primarily plays a permissive role in humans in vivo in regulating endocrine, immune, reproductive and cardiovascular functions, mainly in energy-deficient states (16). It would appear that hypoleptinemia could be linked to pancreatic cancer due to weight loss, but the association persisted after adjusting for changes in BMI and/or weight loss. Thus, the association could also occur independently of weight loss and possibly reflects an underlying association of hypoleptinemia with insulin resistance as previously demonstrated (16).

This is the first study demonstrating the existence of adiponectin in PC and exploring simultaneously the role of adiponectin and leptin in pancreatic cancer pathogenesis taking into account other risk factors as well as assessing adiponectin and leptin in relation to tumor markers and PC stages. Due to the cross-sectional nature of this study, causality can not be inferred. Cohort studies are generally considered to be superior to case-control investigations, but are extremely difficult to undertake in PC due to the rarity of the disease. Pancreatic cancer is a challenge to study given its rarity and the usually late stage at diagnosis. Case-control studies with blood collection are thus appropriate for PC, a rare disease state but any cohort and/or mechanistic studies could potentially add useful perspectives. We included hospital controls which were carefully matched to cases, and with admission diagnoses that are not known to be related with the principal exposure variables, namely hormone levels. Despite the rarity of PC in the general population, we implemented an appropriately powered study, which, despite the modest size, was sufficiently large to generate statistically significant associations with the above variables.

In conclusion, our study demonstrated that low leptin, but high adiponectin levels, are independently associated with PC at diagnosis, even after controlling for anthropometric, lifestyle, and medical history factors. Furthermore, this is the first investigation to demonstrate

that both AdipoR1 and AdipoR2 are expressed in pancreatic tumor tissue. Further research should be conducted to determine if adiponectin and/or leptin might be useful as tumor markers for PC. The association of adiponectin with PC appears to be a part of a compensatory response to insulin resistance and/or changes in BMI in response to the disease and needs to be explored further. Biologic experiments and randomized experimental studies in animal models and in humans are needed to frame a reliable interpretation of such associations. Our data need to be replicated in other populations and the mechanisms underlying the role of adiponectin and leptin in pancreatic cancer require further investigation. Moreover, prospective studies should be conducted, when possible, to determine if elevated adiponectin and low leptin levels are simply markers for pancreatic cancer, causally implicated in PC, and/or whether adipokine concentrations are altered in response to the disease.

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Table 1

Descriptive Characteristics of pancreatic cancer cases (n=81) and control subjects (n=81).

Categorical Variables	Cases	Controls	p-value
Total	81	81	(50.0)
Gender, n (%)			
Male	49	49	(60.5)
Female	32	32	(39.5)
Reason for Hospitalization, n (%)			
Hip Joint Replacement	-	17	(21.0)
Knee Joint Replacement	-	8	(9.9)
Injuries or fractures	-	22	(27.1)
Cataract Operation	-	18	(22.2)
Hernia Operation	-	10	(12.4)
Sciatica	-	6	(7.4)
Pancreatic Cancer Stage, n (%)			
I	0	-	(0)
II	8	-	(9.9)
III	32	-	(39.5)
IV	41	-	(50.6)
History of Diabetes, n (%)			
No	63	74	(77.8)
Yes	18	7	(22.2)
Chronic Period of Diabetes Diagnosis, n (%)			
No Diagnosis	63	74	(77.8)
Present at Cancer Diagnosis	2	1	(2.5)
1-5 years	7	2	(8.6)
5+ years	9	4	(11.1)
Family History of Pancreatic or GI Cancer, n (%)			
No	69	78	(85.2)
Yes	12	3	(14.8)
Alcohol Consumption (glasses/day), n (%)			
0	25	28	(30.9)

Categorical Variables			
	Cases	Controls	p-value
<1	25 (30.9)	27 (33.3)	0.08
1	12 (14.8)	10 (12.4)	
2	5 (6.2)	6 (7.4)	
3	14 (17.3)	10 (12.4)	
Smoking History, n (%)			
Non-Smoker	26 (32.1)	38 (46.9)	0.04
Ex-Smoker	2 (2.5)	4 (4.9)	
Current Smoker	53 (65.4)	39 (48.2)	
Smoking Frequency, packs/day, n (%)			
0	26 (32.1)	38 (46.9)	0.01
½	10 (12.4)	11 (13.6)	
1	24 (29.6)	24 (29.6)	
1+	21 (25.9)	8 (9.9)	
Adiponectin Quartiles, n (%)			
1	15 (18.5)	20 (24.7)	<0.0001
2	10 (12.4)	20 (24.7)	
3	16 (19.8)	21 (25.9)	
4	40 (49.4)	20 (24.7)	
Leptin Quartiles, n (%)			
1	43 (53.1)	20 (24.7)	0.01
2	26 (32.1)	20 (24.7)	
3	7 (8.6)	21 (25.9)	
4	5 (6.2)	20 (24.7)	
Continuous Variables			
	Cases Mean(SD)	Controls Mean(SD)	p-value
Age (years)	69.0	70.1	0.42
Initial Weight [†] (kg)	80.0	75.1	0.01
Weight at Interview (kg)	76.5	74.9	0.37

Categorical Variables	Cases	Controls	p-value
Height (m)	1.70 (0.08)	1.72 (0.08)	0.18
Initial BMI [†] (kg/m ²)	27.7 (4.4)	25.3 (3.9)	<.001
BMI at Interview (kg/m ²)	26.5 (4.2)	25.4 (4.0)	0.06
CEA (ng/ml)	19.3 (7.7)	1.95 (1.0)	<.0001
CA 19-9 (U/ml)	2463.7 (1535.6)	15.9 (8.5)	<.0001
CA 72-4 (U/ml)	38.3 (16.1)	3.7 (3.0)	<.0001
Adiponectin (µg/ml)	14.0 (10.6)	8.6 (4.9)	<.0001
Leptin (ng/ml)	8.07 (9.1)	14.8 (12.2)	0.0001

[†]Initial weight and BMI refer to weight and BMI one-year prior to interview and pancreatic cancer at diagnosis for cases and one-year prior to interview and enrolment in the study for controls.

Table 2
Odds ratios and 95% confidence intervals for pancreatic cancer risk in relation to adiponectin/leptin by control-defined quartiles

	Cases (n)/ Controls (n)	Crude OR ^a (95% CI)	P-value	P-trend	Adjusted OR ^b (95% CI)	P-value	P-trend
Control vs. Pancreatic Cancer cases	Adiponectin Quartile						
	1	15/20	1.00		1.00		0.01
	2	10/20	0.66 (0.24, 1.82)	0.43	0.56 (0.18, 1.72)	0.31	
	3	16/21	1.02 (0.40, 2.62)	0.97	1.57 (0.54, 4.54)	0.41	
	4	40/20	2.66 (1.11, 6.34)	0.03	2.81 (1.04, 7.59)	0.04	
	Leptin Quartile						
	1	43/20	1.00		1.00		0.0002
	2	26/20	0.61 (0.28, 1.34)	0.21	0.82 (0.33, 2.03)	0.67	
	3	7/21	0.16 (0.06, 0.43)	0.0003	0.15 (0.05, 0.48)	0.001	
	4	5/20	0.12 (0.04, 0.36)	0.0002	0.16 (0.05, 0.54)	0.003	

^aOR adjusted for age and gender

^bOR adjusted for age, gender, date of diagnosis, BMI at one year prior to interview, history of diabetes mellitus, family history of pancreatic or gastrointestinal cancer, alcohol consumption, smoking status, and adiponectin/leptin concentrations

Table 3
Serum adiponectin and leptin levels (mean ± standard deviation) among pancreatic cancer patients in different prognostic stages

	Pancreatic Cancer Stage				P-value
	I (n=0)	II (n=8)	III (n=32)	IV (n=41)	
Adiponectin	-	15.38 ± 3.20	12.11 ± 1.66	15.20 ± 1.83	0.43
Mean ± SD					
Leptin	-	10.41 ± 4.53	5.89 ± 1.25	9.32 ± 1.50	0.21
Mean ± SD					

Table 4

Descriptive characteristics and adiponectin receptor expression of individuals providing tissue samples.

Pancreatic Cancer Patients (N=16)		
Age, mean (SD)	59.1	(11.0)
Female, n (%)	5	(31.3)
Stage, n (%)		
II	9	(56.3)
III	6	(37.5)
Unknown	1	(6.3)
Cancer Type, n (%)		
Adenocarcinoma not further classified	6	(37.5)
Ductal adenocarcinoma	10	(62.5)
Differentiation, n (%)		
Poorly differentiated	2	(12.5)
Moderately differentiated	13	(81.3)
Unknown	1	(6.3)
AdipoR1 Expression, n (%)		
None or marginal	2	(12.5)
Positive or strong positive	14	(87.5)
AdipoR2 Expression, n (%)		
None or marginal	1	(6.3)
Positive or strong positive	15	(93.7)