Obesity and Fatty Pancreatic Infiltration Are Risk Factors for Pancreatic Precancerous Lesions (PanIN)

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**Abstract**

**Purpose:** The roles of intravisceral and subcutaneous fat are unknown, and the prevalence of precancerous lesions in obese patients was never evaluated. This study aims to assess the frequency and severity of pancreatic intraepithelial neoplasia (PanIN) and to correlate pathologic findings with metabolic abnormalities, type of fat, and fatty pancreatic infiltration.

**Experimental Design:** Normal pancreatic tissue from surgical specimens was analyzed. Fatty infiltration and fibrosis in intraductal and extracellular locations and PanIN lesions were assessed. General characteristics were collected: body mass index (BMI), diabetes, and tobacco intake. Liver steatosis and subcutaneous and intravisceral fat were assessed by CT scan (ImageJ software).

**Results:** Of note, 110 patients were included [median age, 53.8 (17–85) years]. Arterial hypertension, diabetes, and tobacco intake were found in 19%, 9%, and 23%, respectively. Median BMI was 24 (16–37); BMI < 25: 45%, 25 ≤ BMI ≤ 30: 24%, ≥ 30: 11%. Overall, PanIN lesions were found in 65% (type I, II, and III PanIN in 62%, 38%, and 1%, respectively). Fibrosis and fatty pancreas (intra- and extralobular locations) were found in 1% and 24% and in 30% and 51%, respectively. A correlation was observed between PanIN lesions and fatty pancreas [extralobular (0.01) and intralobular (<0.001)] and intralobular fibrosis (0.003), high BMI (P = 0.02), and subcutaneous (P = 0.02) and intravisceral fat (P = 0.02). The number of PanIN lesions was correlated with intravisceral fat (r = 0.22, P = 0.04), but not with subcutaneous fat (r = 0.14, P = 0.22).

**Conclusions:** Obesity (especially android obesity) and pancreatic fatty infiltration are risk factors for pancreatic precancerous lesions. *Clin Cancer Res; 21(15): 3522–8. ©2015 AACR.*

**See related commentary by Wang et al., p. 3369**

**Introduction**

The role of overweight and obesity as a risk factor of pancreatic cancer was previously debated. It is now well established that up to 20% of all cancers can be attributed to obesity (1). In epidemiologic data, a large American cohort, encompassing 19-pooled prospective studies, encompassing 1.46 million white adults, confirmed that the relative risk of mortality due to cancer increases in obese patients who never smoked [body mass index (BMI)=30 kg/m\textsuperscript{2}] and was assessed to 1.34 (16–37; BMI < 25: 45%, 25 ≤ BMI ≤ 30: 24%, ≥ 30: 11%). Overall, PanIN lesions were found in 65% (type I, II, and III PanIN in 62%, 38%, and 1%, respectively). Fibrosis and fatty pancreas (intra- and extralobular locations) were found in 1% and 24% and in 30% and 51%, respectively. A correlation was observed between PanIN lesions and fatty pancreas [extralobular (0.01) and intralobular (<0.001)] and intralobular fibrosis (0.003), high BMI (P = 0.02), and subcutaneous (P = 0.02) and intravisceral fat (P = 0.02). The number of PanIN lesions was correlated with intravisceral fat (r = 0.22, P = 0.04), but not with subcutaneous fat (r = 0.14, P = 0.22). In multivariate analysis, PanIN lesions were associated with intralobular fibrosis [OR, 5.61; 95% confidence interval (CI), 1.18–42.99] and intralobular fat (OR, 17.86; 95% CI, 4.935–88.12).

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Translational Relevance

Obesity is now well known as a risk factor of pancreatic cancer. Adipose tissue, particularly visceral fat, is known to play a key role in the metabolic dysfunctions as a consequence of obesity. In addition, obesity causes fat infiltration of organs. Pancreatic precancerous lesions (PanIN) are rare in normal pancreatic tissue and are of a low grade of dysplasia. In this study, we assessed for the first time the presence and the severity of PanIN lesions according to obesity and fatty pancreatic infiltration in a large series of patients. Data were assessed by morphological and pathological analyses. These lesions were frequent and severe, and probably account for the increasing incidence of pancreatic cancer in obese patients. The clinical consequences of these findings should be considered within the framework of the current debate over obesity and pancreatic cancer. The association between android obesity and fatty pancreatic infiltration should be considered as a major risk factor of pancreatic cancer. Among individuals at high risk for pancreatic cancer, a more cautious screening probably should be performed in obese patients with fatty pancreas.

The aim of this study was to characterize the frequency and the severity of pancreatic intraepithelial neoplasia (PanIN) in patients with fatty pancreas, to correlate pathologic findings with metabolic abnormalities, hepatic steatosis, tobacco intake, and distribution of fat infiltration (intravisceral or subcutaneous fat).

Materials and Methods

Patients

Before select normal pancreatic tissue, all consecutive patients operated on for benign and small neuroendocrine tumors from 2008 to 2011 were selected. All patients with ductal tumors (pancreatic adenocarcinoma and intraductal pancreatic mucinous neoplasia) were excluded. All specimens presented well-differentiated neuroendocrine tumors, grade 1 or 2, according to the 2010 WHO classification. An Institutional Review Board (CEERB, comité d’éthique en recherche biomédicale du Groupe hospitalier universitaire Nord) approval for the study design and the ethical measures were obtained. The ethical committee reviewed all the written consents.

Pathologic examination

The normal pancreatic parenchyma was analyzed at least 2 cm apart from the tumor and downstream of the tumor to minimize the risk of chronic obstructive pancreatitis lesions. All hematoxylin–eosin–safran-stained slides of pancreatic tissue were retrospectively analyzed by light microscopy by two investigators (A. Couvelard and V. Rebours) for each case, both blinded to clinical and pathologic data, using ImageJ free-ware (http://rsb.info.nih.gov/ij/).

PanIN lesions were graded according to criteria established by the National Cancer Institute–sponsored Pancreas Cancer Think Tank (6) into PanIN-1 (flat, papillary or micropapillary epithelial lesions with mild dysplasia), PanIN-2 (epithelial lesions with moderate dysplasia), and PanIN-3 (epithelial lesions with high grade dysplasia; ref. 7). The extent and distribution of fibrosis were evaluated using the score established by Klöppel and colleagues (8) in intra- and extralobular locations. This score (0–3) was defined as the presence of connective tissue (0, no connective tissue; 1, mild deposits of fibrosis; 2, moderate; or 3, severe amounts of fibrosis) within the interlobular space and into the lobules. Samples with a score >3 were considered at high degree of fibrosis. The same score was adapted to quantify the fat infiltration (ranging from 0 to 2). Perilobular fatty infiltration was defined as the presence of adipocytes within the interlobular space and was scored as follows: 0, no fatty infiltration; 1, some adipocytes; and 2, numerous adipocytes separating the lobules. Intralobular fatty infiltration was scored as 0, no or rare adipocytes in some lobules; 1, scattered adipocytes among most of the lobules; and 2, numerous adipocytes among most of the lobules forming clusters of more than 10 cells.

CT scan analysis

Within 4 weeks before surgery, all patients had a preoperative multiphasic (nonenhanced, arterial, pancreatic parenchymal, and portal venous phases) multidetector CT scan performed for all patients with a General Electric CT Light Speed VCT 64 4.3 (GE Healthcare). It included an initial nonenhanced acquisition with a section thickness of 5 mm, at the level of the umbilicus with the patient in the supine position. Images were saved as DICOM images for analysis. By way of this imaging, radiologic data were assessed by two investigators (V. Rebours and G. d’Assignies), both blinded to clinical and pathologic data, using ImageJ free-ware (http://rsb.info.nih.gov/ij/).

The total fat area (TFA) and the visceral fat area (VFA) were evaluated by measuring pixels with densities in the –190 to –30 Hounsfield units range after delineating the total and visceral compartments and computing the cross-sectional area of each in cm2, as previously reported (9). The subcutaneous fat area (SFA) was obtained by subtracting VFA from TFA. The –190 to –30 Hounsfield units range density allows to measure only fat, excluding bone, muscle, and other intraabdominal organs such as liver, spleen, or small bowel, each having a density fat higher or lower (tutorials available at http://rsbweb.nih.gov/ij/docs/examples/index.html); Fig. 1.

The liver steatosis was assessed by CT scan. The liver density measurement was performed on nonenhanced slices by using region of interest (ROI) as large and homogeneous as possible, placed on the liver for three measurements (one in the left lobe and two in the right lobe), avoiding liver vessels and artifact zones. Liver steatosis was assessed by averaging the three ROI measurements and considered when the mean was >48 Hounsfield units, as reported previously (10).

Data collection

Clinical, radiologic, and pathologic data were obtained from a prospective database with additional retrospective medical records reviewed when necessary. Data recorded included: (i) preoperative clinical characteristics (age at surgery, BMI, diabetes mellitus, smoking status, and arterial hypertension); (ii) radiologic data (liver steatosis assessment, the subcutaneous, and intravisceral fat area (percentage of the total area); and (iii) the pathologic features (number and grade of PanIN lesions, fibrosis, and fat infiltration in intra- and extra-pancreatic lobular locations).
Clinical definitions
Smoking status was categorized as smokers if patients had smoked for at least 2 years (current and ex-daily smokers) and nonsmokers. The number of cigarettes smoked per day and duration of smoking were recorded and expressed as pack-years (pack-years of smoking were calculated at the baseline examination as number of cigarettes per day multiplied by number of years of smoking divided by 20).

The BMI was defined as weight (kg) divided by height squared (m²). Overweight and obesity were defined as BMI ≥ 25 to 29.9 kg/m² and BMI ≥ 30 kg/m², respectively.

Diabetes mellitus according to WHO criteria was defined as a whole venous blood fasting glucose concentration recorded ≥ 126 mg/dL (6.99 mmol/L) at least two determinations or >11 mmol/L, post-prandially, at one determination. Insulin requirement was defined by the inefficacy of adequate diet and oral drugs in preventing hyperglycemias.

Statistical analysis
General characteristics were expressed as median and range or percentages. Comparisons of general characteristics, clinical features, morphologic characteristics, and pathologic data were performed using the Kruskall–Wallis test for continuous data and the χ² test or the Fisher exact test for categorical data. A Spearman correlation coefficient was used to search for correlation between continuous variables. A multidimensional analysis was performed using a logistic regression analysis to search for risk factors of PanIN lesions. The stepwise selection option was used. P values below 0.20 were considered as significant as level of entry in the model. Data were analyzed with the SAS 9.1 statistical software for Windows (SAS Institute Inc.). All statistical tests were two-sided. The critical level of statistical significance was set at a P value of <0.05.

Results
Characteristics of the patients and indications for pancreatic surgery
One hundred and ten consecutive patients were included between 2008 and 2011. Women represented 57% (n = 63) of the patients. All patients were operated on for well-differentiated neuroendocrine tumors, grade 1 (n = 69, 63%) or grade 2 (n = 41, 37%). Surgical procedure was a Whipple procedure (n = 61, 55%), left pancreatectomy (n = 30, 28%), or median
pancreatectomy (n = 19, 12%). The median age at surgery was 53.8 (17–85) years. Arterial hypertension, diabetes, and tobacco intake were found in 19% (n = 19), 9% (n = 10), and 27% (n = 25), respectively. The median tobacco consumption was 20 (1–60) pack-years. The median BMI was 24 [16–37; BMI < 25: 56% (n = 50), 25 ≤ 30: 30% (n = 27), ≥ 30: 13% (n = 12)].

Pathologic data
The median surface of pancreatic tissue, evaluated per specimen, was 7.5 cm$^2$ (1.5–25.5). Fibrosis in intra- and extralobular location was observed in 24% (n = 27) and 1% (n = 1), respectively. Fatty pancreas infiltration was found in 51% (n = 56) of the patients in intralobular site [score 0, n = 54 (99%); score 1, n = 38 (62%); score 2, n = 18 (17%)] and in 30% (n = 33) in extralobular site [score 0, n = 77 (70%); score 1, n = 20 (18%); score 2, n = 13 (12%)]. Figs. 2 and 3.

PanIN lesions were found in 65% of the patients (n = 72). PanIN 1, 2, and 3 were observed in 62% (n = 62), 38% (n = 42), and 1% (n = 1) of the patients, respectively. PanIN 1 and 2 lesions were both present in 34.5% (n = 38) of the patients. The median number of PanIN lesions per patient was 2 (0–27); Fig. 4. After adjustment, it represented one PanIN lesion per 1.86 cm$^2$ of pancreas analyzed in all patients, and one PanIN lesion per 1.3 cm$^2$ of pancreas analyzed in patients with PanIN lesions.

Radiologic characteristics
Median total, visceral, and SFA were 266 cm$^2$ (25–817), 94 cm$^2$ (5–313), and 170 cm$^2$ (12–609), respectively. The median percentage of total, visceral, and SFA (regarding the total surface of the slide) represented: 21% (2–47), 7.3% (0.8–20), and 13% (1.2–31.7), respectively. Liver steatosis was observed in 27%.

Univariate and multivariate analysis
Pancreatic fatty infiltration (intra- or extralobular location) was associated with the percentage of TFA (P < 0.0001), VFA (P < 0.0001), and SFA (P = 0.002).

Fatty infiltration in intralobular site was associated with the percentage of TFA (P = 0.0003), VFA (P < 0.0001), SFA (P = 0.0067), the presence of a liver steatosis (P = 0.04), a higher value of the BMI (22 vs. 25; P = 0.003) and a higher age of the patients (59 vs. 49 years old; P = 0.0002).

Fatty infiltration in extralobular location was associated with the percentage of TFA (P = 0.0012), VFA (P < 0.0001), the presence of a liver steatosis (P = 0.0008), a higher value of the BMI (23 vs. 26; P = 0.0006) and a higher age of the patients (59 vs. 51 years old; P = 0.0004). SFA was not correlated with the extralobular fatty infiltration (P = 0.11).

The risk factors of PanIN lesions are set out in the Table 1. Briefly, the presence of PanIN was associated with fatty infiltration [extra- (P = 0.0051) and intralobular (<0.0001)]. This association was correlated with the fatty pathologic score [extra- (0.01) and intralobular (<0.0001)]. The PanIN lesions were more prevalent in score 2. Other risk factors of PanIN lesions were intralobular fibrosis (P = 0.003), SFA proportion (14% vs. 11.8%; P = 0.02), VFA proportion (8% vs. 5%; P = 0.02), the age of the patients (P = 0.056), and a higher BMI (P = 0.02).

In subgroup analysis, the age of the patients was associated with PanIN-1 (55 vs. 49 years old; P = 0.085), but not with PanIN-2 (55 vs. 51 years old; P = 0.17). BMI was associated with PanIN-2 (23 vs. 25 years old; P = 0.04), but not with PanIN-1 (23 vs. 24 years old; P = 0.0865).

Presence of all types of PanIN, PanIN1, or PanIN2 was not associated with tobacco intake or diabetes.

The number of PanIN lesions was correlated with the severity of liver steatosis (r = 0.25, P = 0.02), the percentage of intravisceral fat (r = 0.22, P = 0.04), but not with the percentage of subcutaneous fat (r = 0.14, P = 0.22) or BMI (r = 0.17, P = 0.1).

In a multivariate model, the presence of PanIN lesions was associated with intralobular fibrosis (OR, 5.61; 95% CI, 1.18–42.99; P = 0.057) and intralobular fatty infiltration (OR, 17.86; 95% CI, 4.935–88.12; P < 0.0001). PanIN2 lesions were associated with intralobular fat (OR, 16; 95% CI, 4.61–75.99; P < 0.0001).

Discussion
In this study, one half of the cohort presented overweight or obesity. The presence of PanIN lesions was associated with fatty infiltration of the pancreas, intralobular fibrosis, a high BMI, subcutaneous and intravisceral fat but was not influenced by tobacco intake or diabetes. Moreover, the number of PanIN lesions was correlated with the percentage of intravisceral fat but not with the percentage of subcutaneous fat or BMI. In a multivariate model, the presence of PanIN lesions (and PanIN-2 especially) was associated with intralobular fatty infiltration.

As previously reported, obesity and overweight are independent risk factors of pancreatic cancer. In the large prospective NIH-AARP Diet and Health study cohort including 1,359 men and 763 women with pancreatic cancer, the etiologic fraction explained by overweight or obesity at any age was 14% overall, 18% in former smokers, and 21% in never smokers. BMI gain of >10 kg/m$^2$ was significantly associated with increased pancreatic cancer risk (4). Little is known about the relation between obesity and pancreatic precancerous lesions. Recently, the influence of obesity was evaluated in patients operated on for

Figure 2.
Fibrosis (black arrows) and fat in intralobular locations.
intraductal papillary mucinous neoplasm (IPMN). High-grade lesions were mainly found in obese patients. Of 254 patients with a BMI of $<35$ kg/m$^2$, 30% had malignant IPMN versus 50% in patients with BMI $>35$ kg/m$^2$ ($P = 0.08$). In branch-duct IPMN, patients with a BMI $<35$ kg/m$^2$ had 12% of malignant IPMN compared with 46% in severely obese patients ($P = 0.01$). Alternatively, in main-duct IPMN, no difference was found in the malignancy rate (11). However, the way by which fat influence the risk of cancer is still poorly understood. Is obesity the indirect reflect of an associated diabetes or glucose intolerance or is there a direct role due chronic inflammation? In an in vivo model of lean and obese mice inoculated with murine pancreatic cancer cells, obese mice developed larger tumors, and significantly more developed metastases. The serum adiponectin concentration correlated negatively and serum insulin concentration correlated positively with tumor proliferation. The intratumoral adipocyte mass in tumors from obese mice was significantly greater than that in lean mice. These data could suggest that both insulin resistance and altered adipokine milieu could lead directly to changes of the microenvironment (12). In our study, the main risk factor of PanIN lesions in univariate and multivariate model was the fatty infiltration, especially in intralobular location (OR, 17.86; 95% CI, 4.935–88.12). This risk factor was independent of the age of the patients and the presence of diabetes. Several studies well demonstrated that the prevalence and incidence of PanIN lesions were higher in cases of chronic pancreatitis compared with normal pancreas. Except in cases of hereditary pancreatitis, in all studies, all the lesions were of low grade of dysplasia; PanIN-2 lesions were exceptionally described and PanIN-3, never (13–16). In this series, PanIN-2 lesions were frequently observed (38% of the patients) and the main and independent risk factor was intralobular fat (OR, 16; 95% CI, 4.61–75.99).

In previous publications, it was demonstrated that PanIN lesions could be associated with a lobulocentric atrophy, surrounding the lesions, and the tissue could be replaced by fat tissue. So the role of PanIN lesions could be debated, if they are due or the cause of fatty changes. In this study, we found that fatty infiltration was not specifically localized around PanIN lesions. This observation suggested that PanIN lesions were due to fatty changes and did not promote the fat infiltration. This suggests a possible role of this type of fatty infiltration in the oncogenic process and points out the possible local role of fat in close contact with PanIN. Moreover, our study highlights the specific role of the intravisceral fat correlated with the number of PanIN lesions.

Obesity is associated with chronic low-grade inflammation (also called meta-inflammation), characterized by abnormal proinflammatory cytokine production, immune activation, disruption in autophagy, promotion of endoplasmic reticulum stress, mitochondrial dysfunction, and increased inflammatory signaling. This chronic inflammation is more pronounced in the visceral than in the subcutaneous fat compartment. Obesity increases the size of adipocytes, leading to their necrosis and a significant accumulation of activated macrophages. The inflammatory mediators, including IL6, IL1, and TNFα, have been implicated in development of insulin resistance and their levels...
increase within adipose tissue. This inflammation is accompanied by an upregulation of anti-inflammatory factors, such as IL10, IL4, and TGFβ (17–22). The inflammatory mediators secreted by macrophages act not only locally, in a paracrine manner, but also contribute to general inflammation. Obesity increases levels of the proinflammatory hormone leptin and decreases its anti-inflammatory counterpart, adiponectin (23). These data probably explain the high prevalence of intralobular fibrosis in our study, correlated with obesity and fat infiltration. Moreover, it highlighted our findings regarding the association of PanIN lesions and predominant intravisceral obesity.

This study supports epidemiologic evidence that obesity is an independent risk factor of pancreatic cancer and suggests that fatty infiltration per se plays a role in pancreatic oncogenesis. Obesity, and especially android obesity with increased intravisceral fat, is a risk factor for precancerous lesions of the pancreas.

Disclosure of Potential Conflicts of Interest

P. Lévy is a consultant/advisory board member for Abbvie and Mayoly Splinder. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions

Conception and design: V. Rebours, S. Gaujoux, P. Ruszniewski, P. Lévy, P. Bedossa, A. Couvelard

Development of methodology: V. Rebours, S. Gaujoux, G. d’Assignies, P. Ruszniewski, A. Couvelard

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): V. Rebours, S. Gaujoux, G. d’Assignies, A. Sauvanet, P. Ruszniewski, A. Couvelard

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Writing, review, and/or revision of the manuscript: V. Rebours, S. Gaujoux, A. Sauvanet, P. Ruszniewski, P. Lévy, A. Couvelard

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