INTERACTIONS BETWEEN THE EXOCRINE AND ENDOCRINE PANCREAS

PhD Thesis

Gábor Zsóri, MD

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Supervisor: László Czakó, MD, DSc

First Department of Medicine

University of Szeged

Szeged

Hungary

Doctoral School of Theoretical Medicine

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INTRODUCTION

The exocrine and endocrine pancreata are closely linked both anatomically and physiologically. Pathological conditions in the exocrine tissue can cause an impairment of the endocrine function and vice versa. Pancreatic exocrine insufficiency (PEI) is defined by a deficiency of exocrine pancreatic enzymes resulting in an inability to maintain normal digestion.

Chronic pancreatitis (CP) is the most common etiology of PEI. Gastrointestinal and pancreatic surgical resections, cystic fibrosis, obstruction of the main pancreatic duct (e.g. pancreatic and ampullary tumors), decreased pancreatic stimulation (e.g. celiac disease), or acid-mediated inactivation of pancreatic enzymes (e.g. Zollinger-Ellison syndrome) can lead to PEI. It has been demonstrated to be present in a considerable percentage (10–74%) of patients with diabetes mellitus (DM). PEI has been indicated by direct or indirect pancreatic function tests to be present in about 50% of type 1 and 30–50% of type 2 diabetes mellitus (T2DM) cases. Steatorrhoea was observed in about 60% of patients with diabetes mellitus and fecal elastase <100 μg/g, indicating relevant, severe damage of the exocrine function.

The level of pancreatic elastase-1 (PE-1) has been reported to be lower or not to differ in patients with HbA1c >8% relative to those with HbA1c ≤8%. However, for the proper management of DM and to prevent microvascular complications, a goal should be to maintain HbA1c <7%.

Previous studies have raised the possibility that pancreatic enzyme replacement therapy (PERT) in patients with DM and PEI may improve glucose metabolism, PEI related symptoms, nutritional parameters and incretin response. However, the significance of an exocrine dysfunction in DM has recently been questioned, and no beneficial effect of PERT on the glucose metabolism was observed in patients with insulin treatment for DM, although a reduction in mild and moderate hypoglycemia was demonstrated.

It is a well-known fact that insulin resistance, diabetes, and obesity cause fat accumulation in many organs, including the liver (nonalcoholic fatty liver disease [NAFLD]), pancreas (nonalcoholic fatty pancreas disease [NAFPD]), and pericardium (pericardial adipose tissue [PAT]). The worldwide prevalence of NAFLD ranges widely from 6.3% to 33% with a median of 20%, depending on the kind of assessment methods used. There is a high prevalence of NAFLD in patients with T2DM (64–69%) and dyslipidemia (20–81%). Obesity, T2DM, and dyslipidemia are risk factors for the development of NAFLD. The

incidence rate of prediabetes mellitus (pre-DM) is as high as 93.3% in NAFLD, so pre-DM is a more important predictor of NAFLD than metabolic syndrome. If left untreated, NAFLD may progress through steatohepatitis to cirrhosis and hepatocellular carcinoma.

NAFPD, defined as pancreatic fat accumulation, is a less well-studied phenomenon. Fatty pancreas is a common ultrasound finding with increased echogenicity of the parenchyma due to fat accumulation. Previous studies have suggested a 16%–35% prevalence of fatty pancreas in the general population. It seems that age, obesity, hyperglycemia, and dyslipidemia are risk factors for NAFPD. It may increase the risk for the development of metabolic syndrome by causing inflammation, impaired pancreatic beta cell function, and finally hyperglycemia. This relationship may explain the presence of T2DM in 6.9–12.6% of patients with NAFPD.

It has been demonstrated that obesity may lead to pancreatic ductal adenocarcinoma through NAFPD. Evidence suggests that NAFPD plays a role in T2DM, pancreatic exocrine dysfunction, acute pancreatitis, pancreatic cancer, and the formation of pancreatic fistulas after pancreatic surgery.

NAFLD and NAFPD are associated with each other because pancreatic fat formation is related to NASH and is a significant predictor of the presence of NAFLD. The elevation of liver transaminases may suggest the presence of NAFLD or NASH. Ultrasonography (US) and transient elastography are currently the most appropriate imaging modality for NAFLD screening, and liver biopsy is the ‘‘gold standard’’ for characterizing liver histology in patients with NAFLD. In contrast to the liver, no biochemical marker is available for diagnosing NAFPD. Further, as the pancreas is a retroperitoneal organ, a pancreatic biopsy is more cumbersome and may be accompanied by more sampling errors and complications compared with a liver biopsy. Visualizing the pancreas by US is more difficult, and the sensitivity and specificity of US in detecting NAFPD are hampered by obesity and bloating. In prediabetic and T2DM patients, the amount of PAT is significantly higher compared with that in normoglycemic patients. Previous reviews have demonstrated that, besides epicardial adipose tissue, PAT is another risk factor for the development of cardiovascular disease in T2DM patients.

AIMS

I. to assess the possible relationship between T2DM with poor glycemic control (HbA1c ≥7.0%), an exocrine pancreatic insufficiency and alterations in pancreatic morphology;
II. to evaluate the incidence rate of NAFLD, NAFPD and PAT size, and the effect of metformin therapy on NAFLD, NAFPD and PAT in new-onset T2DM (NODM);
III. to provide an overview of the current concepts of PEI in diabetes mellitus by performing a systematic review.

PATIENTS AND METHODS

The thesis is based on two clinical studies and a systematic review.

I. Prevalence of exocrine pancreatic insufficiency in type 2 diabetes mellitus with poor glycemic control.

Consecutive patients with T2DM followed-up in the diabetes outpatient clinic of the First Department of Internal Medicine, University of Szeged were prospectively recruited into the study between April 1, 2012 and June 30, 2013. Fasting blood glucose and serum HbA1c level were measured at the time of recruitment. The patients were divided into two groups, depending on the serum level of HbA1c: Group A: patients with poor glycemic control (glycosylated hemoglobin [HbA1c] ≥7%), and Group B: patients with good glycemic control (HbA1c <7%). The pancreatic exocrine function was evaluated via the measurement of fecal PE-1, and morphological examinations were performed on the pancreas.

The diagnosis of DM was made in accordance with the criteria of the American Diabetes Association (ADA) [14]. CP was diagnosed only when both the morphological and functional diagnostic criteria were fulfilled. Cases with type 3cDM, i.e. diabetes secondary to exocrine pancreatic diseases, were excluded. The patients were not operated on the pancreas. Fecal PE-1 was determined through the use of monoclonal antibodies (ScheBo Biotech AG, Giessen, Germany). Abdominal US and computer tomography (CT) were performed to detect the characteristic morphological features of CP.

II. Evaluation the incidence rate of NAFLD, NAFPD and PAT size, and the effect of metformin therapy on NAFLD, NAFPD and PAT in NODM.

The diagnosis of T2DM was made in accordance with the ADA criteria. NODM is defined as DM diagnosed within the past 1 month before the date of enrollment. Patients were only on a low-carbohydrate diet and received no hypoglycemic agents before inclusion. Exclusion criteria consisted of any pancreatic, liver, or cardiovascular disease, inherited disorders of fat metabolism, pregnancy, malignant disease, antidiabetic medication, or alcohol consumption in patients' medical records. NODM patients received no other new drugs beyond 1000 mg metformin twice daily after inclusion. However, a hypolipidic diet was recommended to patients with elevated lipid levels.

The follow-up period was 4 months. CT and laboratory tests (serum triglyceride, cholesterol, insulin level, fasting blood glucose, and HbA1c) were performed before the beginning of metformin therapy and 4 months afterward. Homeostatic model assessment-estimated insulin resistance (HOMA-IR) was also calculated. PAT size and the amount of fat in the pancreas and liver were determined by X-ray attenuation rate during unenhanced CT examination (Hounsfield unit [HU]). Each region of interest (ROI) in the liver, pancreas, and spleen was a round area of 1.0 cm2 as a marker of the degree of attenuation. In the case of PAT, measurements were performed in one dedicated slice at the junction of the inferior vena cava and right atrium. ROIs were identified in segment VII of the liver, along the diaphragmatic surface of the spleen and in the body of the pancreas. Mean density was calculated, and General Electric Centricity PACS software was used to determine the values. NAFLD and NAFPD were defined when the liver-to-spleen or pancreas-to-spleen attenuation ratio was <1.

III. Current concepts of PEI in diabetes mellitus. Systematic review.

The systematic review was conducted following the preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. A systematic search was performed in 3 databases, Pubmed, Embase and Cohraine Library. The search included the following MESH terms: "diabetes mellitus" AND "pancreatic function" OR "pancreatic exocrine insufficiency" OR "fecal elastase" OR "secretin" OR "cholecystokinin" OR "steatorrhea" or "pancreatic enzyme replacement therapy". The search was limited to

human data and to full text English articles if appropriate. The latest date searched was conducted on the 31th of January 2018.

Selection of the studies was conducted by two investigators (Gábor Zsóri and László Czakó) separately. Clinical studies were eligible provided that they reported the data of pancreatic exocrine function in adult patients suffering from type 1 and 2 diabetes mellitus. Publications about type 3cDM were excluded. Duplicates, repeated publications, publications available only in abstract form, and review papers were excluded. Moreover, articles with inappropriate study design and patient inclusion criteria were also excluded from this systematic review. Remaining studies were further analyzed in full text. The reference list of obtained articles was also checked for additional articles. If differences were found in the reviewer's judgment, then a committee of three other researchers was invited to draw a conclusion.

RESULTS

I. Prevalence of exocrine pancreatic insufficiency in type 2 diabetes mellitus with poor glycemic control

A total of 101 type 2 diabetic patients were included into the study: 59 (25 male, 34 female, mean age: 63.6±10.4 years, range: 42–89 years) in Group A and 42 (22 male, 20 female, mean age: 57.1±11.2 years, range: 30–83 years) in Group B. 2 patients had autoimmune disorders, both suffered from rheumatoid arthritis, one in Group A and one in Group B. In group A 12 patients were on oral antidiabetic therapy, while the other 47 patients received insulin treatment. The same in Group B were 23 and 15, respectively, and the rest 4 patients were on diet only. The range of HbA1c was 7.0–11.6% in Group A and 5–6.9% in Group B. The body mass index (BMI) of the patients were comparable in the two groups ($p=0.278$). However, the mean age was significantly higher ($p<0.008$), and the duration of DM was significantly longer ($p<0.006$) in Group A as compared to Group B.

The fecal PE-1 concentration in Group A was normal in 45 patients (76.3%), while 11 (18.6%) exhibited a mild and 3 (5.1%) a severe PEI. In Group B, the PE-1 concentration was normal in 39 subjects (92.9%), while 3 (7.1%) displayed a mild PEI, and none a severe insufficiency. The prevalence of abnormal PE-1 concentration was significantly different between Group A and B (23.7% vs. 7.1%; $p=0.033$). The PE-1 level was significantly lower

in Group A than in Group B (385.9±171.1 vs. 454.6±147.3 μg/g, p<0.04, odds ratio [OR]=4.0). The PE-1 level was not correlated with HbA1c (r=-0.132, p=0.187), the duration of DM (r=-0.046, p=0.65), age (r=0.010, p=0.921), or BMI (r=0.203, p=0.059).

II. Evaluation the incidence rate of NAFLD, NAFPD and PAT size, and the effect of metformin therapy on NAFLD, NAFPD and PAT in NODM

Seventeen patients with NODM (male: 8; female: 9; mean age: 55.3±10.8 years; BMI: 31.8±5.1 kg/m2) were involved in this prospective study. The control group comprised 10 healthy subjects (male: 6; female: 4; mean age: 53.9±13.8 years; BMI: 30.0±4.7 kg/m2) without DM, without the presence of any pancreatic, liver, or cardiovascular disease, or history of alcohol consumption and matched for age, sex, BMI, and serum lipids

Fasting blood glucose, HbA1c, serum insulin and cholesterol, and HOMA-IR decreased significantly after metformin therapy compared with the baseline values. However, serum triglyceride and BMI did not change.

NAFLD was diagnosed in 64.7% (11 out of 17) of the patients with NODM and in 10% of the control subjects based on the diagnostic criteria. The amount of fat in the liver was significantly higher in NODM patients with NAFLD than in those without it (25.2±12.7 vs. 55.6±9.3 HU). The serum cholesterol level was significantly higher in patients without NAFLD as compared with patients with the disease in the NODM group (p=0.002). BMI, serum triglyceride, fasting blood glucose level, HbA1c, and liver enzymes were not significantly different between patients with and without NAFLD.

The radiation absorption of the liver was significantly lower in patients with NODM compared with the control group (32.3±17.7 vs. 53.1± 8.3HU [p =0.001]) and significantly increased after metformin therapy compared with the baseline values (32.3±17.7 vs. 47.3±12.1 HU [p=0.026]). Only six patients (35.3%) had NAFLD after the 4-month metformin therapy according to the diagnostic criteria.

NAFPD was diagnosed in 82.3% (14 out of 17) of the patients with NODM and in 20% of the control subjects based on the diagnostic criteria. The amount of fat in the pancreas was significantly higher in the NODM patients with NAFPD than in those without it (30.2±6.9 vs. 45.4±3.9 HU [p<0.001]). The radiation absorption of the pancreas was significantly lower in the patients with NODM compared with the control group (34.0±7.9 vs. 39.4±7.8 HU [p=0.04]) but did not change significantly after the 4-month metformin treatment (34.0±7.9 vs. 37.7±10.2 HU [p=0.178]).

PAT size was significantly larger in the patients with NODM compared with the control group (2143.1±1036 vs. 1223.9±312.9 mm^2 [p=0.008]) and did not change significantly after the metformin treatment (2143. ±1036 vs. 2048.2±997 mm2 [p=0.798]).

III. Current concepts of PEI in diabetes mellitus. Systematic review

Database searches yielded altogether 1055 articles (EMBASE: 67; PubMed: 701; Cochrane: 287).

Prevalence of exocrine pancreatic insufficiency in diabetes mellitus

There have been numerous reports in recent decades on PEI in patients with diabetes mellitus. In the early studies, pancreatic exocrine function was assessed with the gold-standard method of direct pancreatic function tests (pancreozymin-secretin test). PEI was revealed in 52.4% (18–100%) of the cases. However, these studies were only limited to a small number of patients because direct pancreatic function tests are invasive, time-consuming and expensive. Therefore, a less invasive, cost-effective test was needed to evaluate pancreatic exocrine function in DM. Nowadays, therefore, fecal elastase-1 (FE-1) measurement has become a screening tool in determining PEI. The prevalence of PEI has been demonstrated with FE-1 measurement with an average of 40% (26–74%) in type 1 diabetes and with an average of 27% (10–56%) in type 2 diabetes.

Prevalence of morphologic changes of the exocrine pancreas in diabetes mellitus

Several studies have examined the morphologic changes of the exocrine pancreas in DM. In nearly 50% of type 1 DM patients, the pancreas is atrophic and fibrotic, with fatty infiltration and loss of acinar cells on histological examination. Reduced pancreas size in patients with DM was demonstrated by abdominal US, CT or MRI. Ductal changes are detected by endoscopic retrograde cholangiopancreatography (ERCP) in 76% of diabetics. Interestingly, these ductal changes do not correlate with DM type, DM duration or age.

Pathophysiology of PEI in diabetes mellitus

Pancreas atrophy is a related event in DM and plays a central role in the development of PEI. (1) Insulin has a trophic effect on pancreatic acinar tissue through the insulin-acinar portal system, so its decreased locally high level could lead to pancreatic atrophy. (2) Acute hyperglycemia was demonstrated to inhibit basal and cholecystokinin-stimulated pancreatic enzyme secretion with an insulin-independent mechanism. (3) Pancreatic stellate cells (PSCs) play a pivotal role in pancreatic fibrosis. Hyperglycemia was demonstrated to promote proliferation and activation of PSCs and to stimulate collagen production of PSCs via the protein kinase seCp38 mitogen-activated protein kinase pathway, resulting in pancreatic fibrosis. (4) The islet hormones (e.g. glucagon and somatostatin) can regulate exocrine tissue, so the lack of these hormones causes dysregulation of enzyme synthesis and resultant exocrine insufficiency. (5) Diabetic microangiopathy leads to insufficient perfusion through local microangiopathy, resulting in ischemia of the exocrine pancreas, which could lead to pancreatic fibrosis, atrophy and PEI. (6) Autonomic neuropathy may give rise to impaired enteropathic reflexes and PEI. Moreover, (7) viral infections, (8) autoimmunity, or (9) genetic changes, as single-base deletion in the variable number of tandem repeats containing exon 11 of the carboxyl ester lipase gene could increase simultaneous damage to exocrine and endocrine tissue.

Nowadays the role of signaling proteins in pancreatic inflammation and diabetes induced pancreatic insufficiency is getting more attention. In a previous study the levels of total PKB, p70S6K, 4 E-BP1, ERK1/2, and NF-kappaB in the diabetic pancreas compared to control were significant decreased, however, the phosphorylation of p70S6K1, 4 E-BP1, ERK1/2, and protein ubiquitination were increased significantly compared to control group. Presumable, that these factors are liable for decreased enzyme synthesis and pancreatic atrophy.

Symptoms of PEI in diabetes mellitus

The main clinical symptoms of PEI are due to the maldigestion and malabsorption of fat, including steatorrhea, abdominal pain, flatulence, bloating and weight loss. Although PEI seems to be frequent in DM, data on the occurrence of the symptoms of PEI in diabetes are limited. Gastrointestinal (GI) symptoms are common (27–87%) in patients with type 1 and type 2 DM. In a recent study 24% of diabetic patients had one or more GI symptoms

consistent with a diagnosis of PEI (Bristol stool type 5–7, steatorrhea or weight loss). Among these patients, 42% had a low FE-1, indicating PEI. It can be concluded that FE-1 screening is beneficial in patients with GI symptoms, suggesting the presence of PEI.

Diagnosis of PEI

PEI is suggested by clinical symptoms or poor glycemic control despite an adequate diet, antidiabetic therapy and patient adherence. Determination of FE-1 is the most convenient way to diagnose PEI. Decreased FE-1 concentration has previously been demonstrated to be a sensitive method in moderate and severe PEI (sensitivity: 87% and 95%, respectively) and correlated significantly with the direct pancreatic function test, fat digestion, and the Cambridge severity classification of chronic pancreatitis. PEI can also be diagnosed with a 13C mixed triglyceride breath test by measuring the concentration of 13CO2 in expired air after administering the radiolabeled test meal containing a known amount of fat. Coefficient of fat absorption (CFA) is another gold standard test for PEI, although it has not been evaluated in DM.

Direct pancreatic function tests are considered the gold standard in diagnosing PEI, and they definitely have advantages over indirect tests. However, direct tests are rather time-consuming and expensive to perform, very inconvenient for patients, and only available in a few academic centers.

Therapy of PEI

PERT is applied in PEI to prevent the symptoms of malabsorption, such as steatorrhea, and to provide physiologic nutrition by correcting maldigestion. Only a very limited number of publications have investigated the effectiveness of PERT in PEI associated with diabetes, and the results are contradictory. Recent guidelines recommend a starting dose of PERT to be 50 000 IU lipase per main meal and 25 000 IU per snack, and this may be titrated up according to symptoms. However, recent evidence suggests that even this dose of PERT may not be sufficient to normalize nutrition.

Nutrient-induced glucose-dependent insulinotropic polypeptide (GIP) response is diminished in patients with PEI. PERT has been demonstrated to reverse an impaired GIP response and therefore to restore the incretin effect of fat. This effect of PERT may be beneficial in the glycemic control of diabetic patients with PEI.

However, while diabetic patients with reduced FE-1 may not complain about PEI-related gastrointestinal symptoms, they still might suffer from qualitative fat maldigestion, for example, lack of vitamin D, as has been proposed recently. Furthermore, patients with diabetes mellitus have an increased risk of bone fractures. PERT has been demonstrated to increase serum vitamin D level in diabetic patients with PEI, an effect which would be beneficial to reducing the increased risk of bone fracture.

The currently available evidence is limited to answering the question of whether PERT is efficacious in glycemic control in patients with diabetes and PEI. Without doubt, there is a need for further randomized clinical trials in the field. For the moment, we can only suggest searching for PEI in diabetic patients by looking for abdominal symptoms that may be related to PEI and by analyzing serum nutritional factors and vitamin D level. If the test is positive, a trial of PERT is recommended. The response of abdominal symptoms, serum nutritional factors and parameters of glucose metabolism should be followed. In the case of positive response, long-term PERT is suggested.

NEW RESULTS ESTABLISHED IN THE THESIS

I. Prevalence of exocrine pancreatic insufficiency in type 2 diabetes mellitus with poor glycemic control

1) PEI demonstrated by fecal PE-1 determination is more frequent in T2DM patients with poor glycemic control.
2) The impaired exocrine pancreatic function cannot be explained by an alteration in the size of the pancreas or by pancreatic steatosis.

II. Evaluation the incidence rate of NAFLD, NAFPD and PAT size, and the effect of metformin therapy on NAFLD, NAFPD and PAT in NODM

1) NAFLD, NAFPD, and increased PAT were detected in the majority of patients with NODM.
2) Metformin therapy decreased the amount of fat in the liver in parallel with an improvement in the metabolic parameters and may, thus, be beneficial for preventing the late consequences of NAFLD.

III. Current concepts of PEI in diabetes mellitus. Systematic review

1) PEI is detected in almost 50% of diabetic patients by direct or indirect pancreatic function tests.
2) Morphological changes in the pancreas is revealed in approximately 50% of diabetic patients.
3) Determination of FE-1 is the most appropriate methods to diagnose PEI in symptomatic diabetic patients
4) The currently available evidence is limited to answering the question of whether PERT is efficacious in glycemic control in patients with diabetes and PEI.

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